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Marine Biodiversity of Antarctic hard rock communities: species biomass and energy use

A thesis submitted in accordance with the requirements of
the Open University for the degree of

Doctor of Philosophy

By

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Sponsoring establishment:

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British Antarctic Survey

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August 2017

Dedicated to my family for all your support

Thank you

Look beyond the macrofauna.....



Photograph Terri Souster

The smallest details often tell the greatest
stories; you just have to look in the right way
(Martin A. Buzas, Ecologist)



Photograph Terri Souster

Abstract

This thesis presents the first seasonal study in the Antarctic of shallow water hard rock communities, including surveys to quantify biodiversity and biomass (Ash Free Dry Mass, AFDM). In addition temporal changes in biodiversity since 1998 were evaluated in the same location. To do this all benthic organisms greater than 3mm in size were collected at 6m, 12m and 20m depth from three transects near Rothera Point, Adelaide Island (67° 34' S, 68° 07' W) in the summer and winter of 2015. Organisms were identified and their wet, dry and ash free dry masses obtained. Benthic community structure did not vary seasonally, low metabolic costs due to slowed growth rates, reproduction, development with the ability to cease feeding for the winter months may explain the lack of change between season. Community structure did, however, vary with depth, which agrees with previous studies of shallow water Antarctic hard substratum communities. Comparisons between the 2015 and 1998 survey showed reductions of both biodiversity and organic biomass at all three depths over the intervening 17 years. The greatest change was at 12m where faunal density, diversity, richness and biomass all declined significantly between 1998 and 2015. Ice scour is thought to be the main driver of this change as previous studies have shown an increase in ice scour frequency around 10m depth .

The effects of seasonality on metabolism were also investigated using the five most common marine invertebrates and significant energy transformers *Odontaster validus*, *Sterechinus neumayeri*, *Nacella concinna*, *Heterocucumis steineni* and *Ophionotus victoriae*. Measurements of metabolic rates using closed circuit respirometry were carried out across a size range (juveniles to fully reproductive adults), to represent the population of five locally abundant species during the austral summer and winter. Oxygen consumption of *Sterechinus neumayeri* and *Odontaster validus* was significantly higher (by 39% & 44% respectively) in summer than winter. However, metabolic rates showed no consistent seasonal trends in *Nacella concinna*, and in *Ophionotus victoriae* and *Heterocucumis*

steineni were higher in summer than winter, but only in large individuals which could be due to feeding and reproducing during the summer months. Seasonal metabolic changes were in line with previous studies on Antarctic marine invertebrates.

Having established the metabolic rates of these species, identifying how much organic carbon there is in each and what they eat (diet), it is possible to estimate the energy required within the ecosystem to meet their metabolic demands long term. Molecular methods were employed to advance our understanding of diet. Gut contents from the same five species were analysed using DNA extraction and molecular techniques. This project forms a baseline to understand future changes in Antarctic benthic biodiversity and to analyse energy flows in these communities.

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The highest, driest, windiest, most inhospitable yet the most beautiful, bizarre and exciting continent on earth; this is Antarctica where I was lucky enough to carry out research for my PhD. Rothera Research Station on the Western side of the Antarctic Peninsula had been my place of work and home for three previous Antarctic winters when Prof Lloyd Peck, Dr Simon Morley and Prof Melody Clark offered me the opportunity to return to study benthic marine biodiversity of hard substrata. Their belief that I could achieve the huge amount of research needed in 16 months inspired me and kept me going. Thank you for your support at Rothera and for being there at the end of the phone and email.

The 2015 Antarctic winter Marine Team assisted me both in the water and out on the winter sea ice. PhD research is rarely achieved by an individual and needs a good team, thank you for all your help. Also thank you to Matt Brown, previous Winter Base Commander and Diving Officer for making me the Antarctic diver I needed to be to work on a diving intensive research programme and for being a great friend. Many thanks also to Dr David Barnes for your friendship, guidance, patience, hard work and endless biodiversity debates.

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Chapter 1 – Introduction

Chapter 1 Introduction

Biodiversity and climate change

Biodiversity describes the variation of all life, including not only the vast variety of organisms but also their biogeography and community structure. Changes in biodiversity alter ecosystem processes and thus alter the resilience of ecosystems to environmental change (Chapin, Zavaleta et al. 2000). Ecosystems when highly stressed or disturbed support lower levels of biological diversity (Steudel, Hector et al. 2012). However, “the disturbance hypothesis” suggests that species diversity is maximized when ecological disturbance is neither too rare nor too frequent (Connell 1978, Molino and Sabatier 2001). For example, loss of species richness has been described in Polar Regions due to increased scour from icebergs and increased deglaciation (Smale, Barnes et al. 2008a, Barnes and Souster 2011). Disturbance has always shaped the evolution and ecology of organisms and communities and nowhere is this more apparent than on the iceberg-gouged continental shelves of the Antarctic Peninsula (Smale and Barnes 2008). In the Polar Regions it can take a long time to recover when communities are disturbed (Barnes 2017a) and Conlan and Kvitek (2005) followed faunal recovery of 19 iceberg scours in the Canadian Arctic and suggested that > 10 years is required for scoured communities to recover to background levels. As climate change is affecting all regions of our planet there is an urgent need to understand the effects on biodiversity (Bellard, Bertelsmeier et al. 2012). On a global scale, ocean warming is greatest near the surface; and the upper 75 m warmed by 0.11 (0.09 to 0.13°C) per decade over the period 1971 to 2010 (IPCC 2014). Ocean surface temperatures are projected to rise further over the 21st century under all assessed emission scenarios (IPCC 2014). Extreme climatic events are predicted to increase in frequency and magnitude, but their ecological impacts are poorly understood (Thibault and Brown 2008).

In all assessments of biodiversity, it is vital to have comprehensive baseline data in order to establish the effects of change on the environment (Rogers, Johnston et al. 2012). To assess change in biodiversity, 4 main components can be considered:

- **Genetic biodiversity** – Variety in the number and types of genes within the population of a species and also between species. Genetic diversity provides the building blocks for adaptation in a changing world giving organisms unique traits (Templeton 1994)
- **Species richness** – The number of different species represented in an ecological community (Gotelli and Colwell 2001)
- **Ecosystem diversity** – Variety of ecosystems in a given place. An ecosystem being a community of organisms and their physical interactions with the environment (Sohier 2007)
- **Community-level interactions** – The interactions between two or more species within an ecosystem, which provides trophic balance within that system (Ferrier and Guisan 2006).

Biodiversity assessments can be conducted using any of the components above, or a combination. However to detect the effect of change on an ecosystem, a comprehensive baseline study is required using methodologies and analyses which can be repeated over time. Currently what is required is a detailed quantitative investigation of the biodiversity of shallow water Antarctic hard rock communities, across phyla and organisms >3mm in size. No such detailed baseline data currently exists for future biodiversity comparisons and assessments of change in the Antarctic.

The importance of baseline studies in biodiversity assessments

One of the overriding problems identified is that in many key areas biodiversity are, and remain, to a large extent unquantified. Consequently past changes have not been measured or described (Hogg, Barnes et al. 2011). Thus it is essential to develop such baseline data. This should consist of taxonomic descriptions of the organisms that are there, their abundance and how this varies with time (season and year).

There is a long tradition in ecology of drawing a distinction between the study of pattern and process. However, the abundance of species in the wild is dictated by many factors and not a single process (Clarke 1996). Once richness is established it is then important to be able to evaluate process, which describes how the ecosystem functions. For example analysing how much biomass contributes to describing how much energy is in the system and the potential ways such energy may be utilised.

Biomass is defined as the mass of living organisms (organically bound carbon) in a given area or ecosystem at a given time (Black 2013). Organic biomass is used for growth, reproduction, maintenance and respiration and in benthic marine invertebrates is closely related to surface primary productivity, i.e food supply (Grebmeier, McRoy et al. 1988). However, in some areas where productivity is low, such as the deep sea in Antarctica, the biomass is still high (Thresher, Adkins et al. 2011). By identifying how much organic carbon there is in each species, their metabolic rates and what they eat, it is possible to estimate the energy required within the ecosystem to meet their metabolic demands, long-term. The study of the energy transfer between trophic levels is an important step in understanding how an ecosystem functions and how it might be affected when conditions change. Food web studies have provided insight into the dynamics of biomass partitioning, production and stability in ecosystems, stimulating research into the relative role of bottom-up and top-down processes affecting community structure (Elton 1927, Lindeman 1942, Polis and Winemiller 1996, Post 2002). The benthos in Antarctica is the richest element of the marine food web in terms of numbers of macrospecies (Griffiths 2010), but their roles and interactions are poorly understood although suspension feeders are considered to dominate in the shallows and deposit feeders in deeper waters (Griffiths 2010). Biomass, in many studies along the Antarctic and sub Antarctic coasts, has generally been reported only as wet mass (WM) because of the taxonomic importance of the material collected (Muhlehardt- Siegel 1988). However WM is not an accurate measurement for organic biomass as some benthic invertebrates such as the holothurian *Heterocucumis steineni* contain +/- 80% water (Nicol 1967). Therefore an objective of this study will be to

quantify the amount of organic carbon in terms of ash free dry mass (AFDM) for the shallow water Antarctic hard rock communities, across phyla and organisms >3mm in size, which will be the first biodiversity study using AFDM in the coastal shallows of Antarctica.

Energy production and flow are an important proxy of ecosystem function. Energy flow through a basic heterotrophic ecological unit (i.e. one individual) involves consumption (diet analysis), assimilation (growth, reproduction, maintenance and respiration), egestion and biomass loss from predation. Measurement of an organism's oxygen consumption allows the estimation of its metabolic rate as long as anaerobic metabolism does not play a significant role in energy production. Biomass is maintained and supported by the metabolism of the organisms involved. Metabolism provides the life-sustaining chemical reactions within cells of living organisms. It enables organisms to grow, reproduce, maintain their structures and respond to their environments. Animals require energy for all biological functions. Biotic impacts of environmental change are mediated through physiological processes, including metabolic rate (Dillon, Wang et al. 2010, Pörtner and Farrell 2008). There are studies that show the importance of including data on the physiological flexibility of a species when modeling its vulnerability to extinction from climate change (e.g. Pörtner 2012, Thompson, Brown et al. 2015). To lay down biomass organisms must acquire resources by feeding, process the food, assimilate the absorbed molecules and lay down assimilated material as structure. Therefore another objective of this study will be to examine the metabolic rates of five common benthic marine invertebrates, which are important components of the ecosystem and significant energy conformers. The metabolic rates data, combined with diversity and biomass data, knowledge of trophic ecology of key species could then be used to construct a preliminary energy flow diagram for a shallow rocky ecosystem.

Climate change emerged as one of the major threats to biodiversity during the 1990s. The composition and distributions of marine species are being altered by climate change, and are expected to continue to do so. The first assessment report of the Intergovernmental Panel on Climate Change (IPCC) was completed in 1990 and served as the United Nations framework convention on climate change. The most recent 2015 IPCC report has projected that, “surface temperatures (both land and sea) to rise over the 21st century under all assessed emission scenarios”. It is very likely that “heat waves will occur more often and last longer, extreme precipitation events will become more intense and the oceans will continue to warm and acidify” (IPCC 2015).

The assemblages of species in ecological communities reflect interactions among organisms as well as between organisms and the abiotic environment. It might be predicted, therefore, that rapid climate change or extreme climatic events will significantly alter community composition (Walther, Post et al. 2002). Substantial impacts of climate change on community structure have already been observed in a number of systems. A terrestrial example of an extreme climatic event was seen in the Sonoran desert of the southwestern United States where increases in woody shrub density, extinction of previously common animal species and increases in formerly rare animal species have been attributed to regional climatic shifts (Thibault and Brown 2008). A marine example is that coral reefs during periods of warmer than normal sea temperatures have undergone mass bleaching events whenever sea temperatures have exceeded long-term summer averages by more than 1.0 °C for several weeks (Hoegh-Guldberg 1999). The most severe period occurred in 1998, in which an estimated 16% of the world's reef-building corals died (Brown 1997). Another well known marine example is the El Niño which is the warming of the ocean surface to above average temperatures in the central and eastern tropical Pacific Oceans. Coincident with the summer season in the Peruvian and Ecuadorian coastal areas, the normally cold water of the north-flowing Peru Current is displaced by a warm, southward current associated with a decrease in nutrients and a temporary reduction in

fishing success (Tegner and Dayton 1987). During 1997 and 1998, one of the longest and most severe El Niños on record struck the Galápagos Islands. Water temperatures in the Galápagos Archipelago, normally between 18°C and 23°C, remained elevated around 32°C for nearly 18 months (Oberhuber, Roeckner et al. 1998). This led to a severe reduction in the food supply of the marine iguanas (*Amblyrhynchus cristatus*) and resulted in widespread starvation, as observed during previous El Niños (Laurie 1990).

These examples show that changes in temperature can produce dramatic effects on biodiversity. Thus it is essential to monitor biodiversity and climate change effects both globally and regionally. Of particular importance are those regions of the globe that are changing the fastest such as the poles. In the Antarctic, the Antarctic Peninsula has experienced the most rapid rates of regional climate change (Meredith and King 2005, Barnes, Fuentes et al. 2006). With sea surface summer temperatures rising more than 1 °C, the Western Antarctic Peninsula was a hotspot for regional warming and sea ice loss (Barnes and Souster 2011) in the last half of the 20th century. However Turner, Lu et al. (2016) found that in the last two decades, air temperatures on the WAP have remained stable. There is very little benthic biodiversity baseline data from the WAP, however previous shallow water benthic biodiversity studies on the WAP include, soft sediment biodiversity King Edward Cove, South Georgia (Platt 1979), the seasonality of recruitment in Antarctic sessile marine benthos (Bowden 2005), hard substratum biodiversity research at Deception Island (Barnes, Linse et al 2008), hard substratum biodiversity research at South Orkney Islands (Barnes, Kaiser 2009), the seasonal physiology and ecology of Antarctic marine benthic predators and scavengers (Obermüller, Morley et al. 2010), soft sediment biodiversity research in Potter Cove, King George island (Pasotti, Manini et al 2014) and the *in situ* settlement of benthic fouling communities under future climate change scenarios (Ashton, Morley et al. 2017). These projects have significantly contributed towards the general knowledge of marine biodiversity around the WAP and also provide important data underpinning the experiments

and data interpretation within this study. Another objective of this study will be to provide a more detailed baseline data set for future studies.

The Southern Ocean and the Antarctic marine environment

Antarctica is the coldest, driest and windiest continent on the planet with extreme changes through the seasons. It is often and justifiably described as an extreme environment. Twice the size of Australia, and holding the majority of the Earth's ice on its surface, Antarctica is central to our understanding of the planet's climate and oceanic circulation systems (MacFarling Meure, Etheridge et al. 2006).

Antarctica separated from South America between 24 and 40 million years ago. With the opening of the Drake Passage, the Polar Frontal Zone (PFZ) was formed by the formation of the Antarctic Circumpolar Current (ACC) (Crame 1999) and cooling of the Southern Ocean.

The ACC is one of the world's largest, fast flowing ocean currents, which moves west to east due to the lack of any landmass connecting with Antarctica. This effectively isolates the cold waters surrounding the Antarctic continent (the Southern Ocean) from the warm waters of the Pacific, Atlantic and Indian Oceans. The Southern Ocean, also known as the Antarctic Ocean, is the fourth-largest Ocean, larger than the Arctic Ocean and comprises about 10% of the total world ocean area. Within the ACC is the Polar Frontal Zone (PFZ) or Antarctic Convergence, which is the boundary between cold Antarctic waters flowing north that meet the relatively warmer waters of the Sub- Antarctic (Figure 1.1).

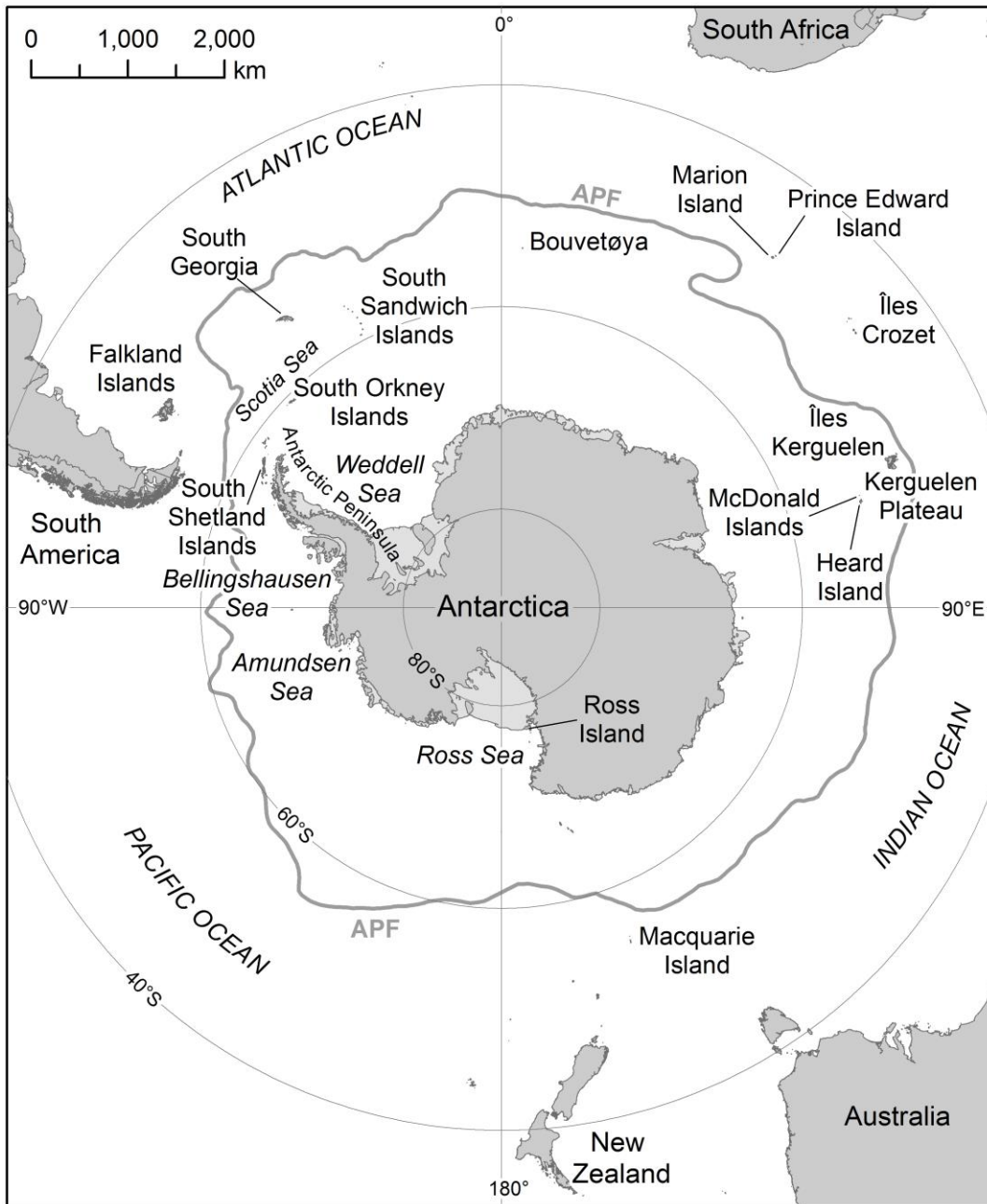


Figure 1.1. The Antarctic continent surrounded by the Southern Ocean showing the Antarctic Polar Frontal Zone (PFZ).

The Southern Ocean is highly seasonal, with a sea ice cover which doubles the size of the continent in winter and decreases rapidly from mid-November to mid-January in the austral summer (Gordon 1981, Thomas 2004). The high latitude also dictates the marked annual variability of daylight from 24 hours of light in summer to 24 hours of dark in winter within the area south of the Antarctic circle (Clarke, Meredith et al. 2008). The start of the summer phytoplankton bloom is generally very predictable and coincides with the onset of stable water conditions (Rozema, Venables et al. 2017), although it may be delayed by the persistence of winter fast ice (Clarke 1988, Riaux-Gobin, Poulin et al. 2011). The Antarctic benthic thermal environment is thus typically viewed as cold and stable, and one where small seasonal or spatial differences in temperature probably have little ecological relevance (Clarke, Griffiths et al. 2009). Thermal stability is a result of the geographical isolation of the Antarctic continent and its waters from the warmer waters of the other oceans to the north by the ACC. However although the Southern Ocean varies little in temperature when compared with temperate oceans, it also has a higher pH and a higher (nearly double in some cases) oxygen content (Routledge 2007). Current estimates of the primary production in the Southern Ocean south of the sub Antarctic front range from 1.2 – 3.5 Gtonne C year⁻¹ (Huntley, Lopez et al. 1991). The Southern Ocean is currently responsible for 40% of the global anthropogenic oceanic carbon uptake (Fletcher, Gruber et al. 2006) and is therefore important in determining atmospheric CO₂ concentration and future climate.

The Antarctic benthic environment is undeniably harsh in our eyes, but it is predictable and stable below the limit of physical disturbance from icebergs (Clarke and Johnston 2003, Barnes and Conlan 2007). Surface water temperature ranges from +1.5 to -1.8°C (Barnes 2017a). From comparison across scales in time and space in Antarctica, the strong seasonal signal in shallow sea temperatures is a notable feature even at the highest latitude sites on the continent, but compared to temperate latitudes it is the lack of variation in shallow sea temperature that is most striking.

The winter sea temperatures at localities within the PF are similar (near freezing), but upper temperatures and thus the annual range vary predictably with latitude. This amounts to 0.2°C annual range/100 km of latitude (Barnes, Fuentes et al. 2006). Studies have shown that with the exception of organisms living in the most shallow water where anchor ice and ice scour are important structuring forces (Smale, Barnes et al. 2008) the benthic community is remarkably rich and stable (Clarke and Johnston 2003).

Biodiversity in the Southern Ocean

Whilst the polar regions were long regarded as areas of low marine diversity, a view which seemed intuitively reasonable given the harshness of the environment, it is now recognized that the diversity of Antarctic continental shelves exceeds that of the Arctic, and is comparable with temperate and even some non-reef tropical shelves (Clarke 2008a). Based on data from the Weddell Sea in the Atlantic sector of the Southern Ocean, the total number of macrozoobenthic species was estimated at 11,000 and 17,000 for the entire Antarctic shelf (Gutt, Sirenko et al. 2004). A cross section of major macro-invertebrate groups shows the species richness of the Antarctic continental shelf is comparable with the shelf faunas of Hawaii or north-west Europe (Clarke 2008). Antarctica has >8% of the world's species in many major groups, and many of these occur nowhere else (Arntz, Gutt et al. 1997). Indeed, certain classes of fauna are more diverse in the Southern Ocean than in lower latitudes, for example over 20% of the total number of sea spiders (pycnogonids) are present in the Southern Ocean (Griffiths 2010) on roughly 11% of the world's continental shelf (Aronson et al. 2007).

In recent years, biodiversity research has significantly increased across the Antarctic continent and the surrounding Southern Ocean (Convey et al. 2014). This growing interest has been driven in part by the realization that any fundamental attempt to understand the diversity of life requires understanding of the polar regions (Chown 2012, Chown, Clarke et al. 2015). The benthic system in Antarctica depends almost entirely upon the short spring/summer phytoplankton bloom. Despite this, the benthic

fauna is surprisingly rich (Clarke and Johnston 2003) (Vausse, Morley et al. in press); however, densities are rather patchy (Arntz, Brey et al. 1992). Antarctic species show significantly wider depth ranges in selected families of the groups Bivalvia, Gastropoda, Amphipoda and Decapoda (Brey, Dahm et al. 1996) which could contribute to patchiness due to a greater area and choice of niches. Echinoderms, in general, are one of the more important taxa in Antarctic benthic ecosystems, constituting 45% of the large epifauna in terms of both numerical abundance and weight. In particular ophiuroids can predominate, representing up to 75% of total numerical abundance and 36% of total mass (Moya, Ramos et al. 2003).

The Register of Antarctic Marine Species (RAMS, October 2010) includes 16,803 taxon names, among which 8,193 are referenced. 88% of the referenced species are benthic (De Broyer and Danis 2011). Exploration of new areas coupled with an emphasis on primary taxonomy have resulted in the description of many new taxa, though continued work in the deep sea coupled with the further application of molecular techniques will undoubtedly add further to documented Antarctic diversity (Clarke 2008a). There are clearly many species in the Southern Ocean still awaiting discovery and description.

Unique marine adaptations and vulnerability to climate change

In addition to high species richness in the Southern Ocean, the isolation and constant cold of the marine environment have allowed the endemic species to evolve some unique adaptations. Certain generalized characteristics seem to be characteristic of living in the extreme cold in the Antarctic and these include:

Growth rates which are 2 – 5 times slower than temperate species (Peck 2016). For example, the Antarctic bivalve *Aequioidia eightsi* grows on average an order of magnitude slower than similar temperate species (Peck, Colman et al. 2000). The Antarctic brachiopod *Liothyrella uva* has an annual growth rate of 0.96 – 2.3 mm yr⁻¹ depending on the age of the animal, this is 2 – 6 times slower than those from temperate species (Peck, Brockington et al. 1997). Regeneration is also dramatically slowed; for example the Antarctic brittlestar *Ophiura crassa* displays high levels of natural arm damage and repair (72%) due to ice damage in shallow water habitats. Regeneration of its arms occurs at approximately 0.16 mm per month; this is the slowest regeneration rate measured in any ophiuroid to date (Clark and Souster 2012).

Reproduction and development are similarly slow. For example, most marine invertebrates at high latitudes, with the exception of one or two species require 18 – 24 months for gametogenesis compared to 6-12 months in temperate species (Grange, Peck et al. 2011). Reproductive output in Antarctic marine species is low compared with temperate species (Arntz, Brey et al. 1994). Fertilisation studies indicated that Antarctic invertebrates require 1-2 orders of magnitude more sperm to ensure optimal fertilisation success. These sperm tended to be long-lived and capable of fertilising eggs 24+ hours after release (Grange 2005). Indeed, Powell, Tyler et al. (2001) found using the optimum sperm concentration for fertilisation success that spermatozoa were capable of fertilising fresh ova for > 90 h in *L. elliptica*, and ~65 h in *N. concinna*.

Larval development in Antarctic marine invertebrates can be 5 – 10 times slower than in temperate species (Peck 2016). Echinoid species from McMurdo Sound (East Antarctica) take 85 -140 hours to hatch compared with 15 – 30 hours for species living at 8 -25°C (Pearse 1969, Bosch, Beauchamp et al. 1987, Powell, Tyler et al. 2001) and some brooding species take over 2 years to complete development (Hain and Arnaud 1992, Peck, Clarke et al. 2006).

This slowed growth and reproduction means that in an area of rapid climate change, the organisms have long generation times and therefore have fewer generations than temperate and tropical species in which to adapt genetically to new conditions.

Life expectancy is long and many Antarctic marine invertebrates live extraordinarily long lives compared with temperate or tropical species. Antarctic krill *Euphausia superba* live for up to ten years whereas temperate krill live on average for just two years (Ikeda and Thomas 1987). The endemic Antarctic clam *Laternula elliptica* can live for 36 years and the bivalve mollusc *Aequiomya edwardsi* (Peck, Colman et al. 2000, Román-González, Scourse et al. 2017) and brachiopods have lifespans of 60 years (Peck, Brockington et al. 1997) .

Polar waters are high in dissolved oxygen because the solubility of oxygen increases at low temperatures. Oxygen availability is also recognized widely as a major factor in determining the impacts of climate change on marine systems (Spicer 2014). A widely cited paper by Peck and Chapelle (1999), found that the maximum size of amphipods across the globe was related to dissolved oxygen rather than temperature or salinity. Giant amphipods may therefore be among the first species to disappear if global temperatures increase and oxygen levels decline. Gigantism in polar benthic marine invertebrates is due in part to the higher oxygen content of the water, but the lower sea temperatures produce lower basal metabolic rates, which also has an effect (Moran and Woods 2012).

For example; among pycnogonid spiders, the leg span of a British species *Pycnogonum littorale* is 20mm (Oxford University Museum of Natural History [OUMNH](#) collection) whereas that of a polar species *Colossendeis wilsoni* may be up to 750mm (OUMNH collection). The giant isopod *Glyptonotus antarcticus* in the Antarctic grows up to 20cm in length and weighs 70g whereas isopods in coastal temperate and tropical water are just a few centimeters in length (Kaiser and Attrill 2005). If oxygen is important in setting size limits, then climate change is a potentially serious threat to specialist groups of species functioning with narrow oxygen safety margins. Polar marine species living permanently at temperatures near zero appear to have among the poorest abilities to respond to changes in environmental temperature (Peck, Morley et al. 2014). As Antarctic marine animals are so well adapted to cold stable conditions, they are very vulnerable to environmental change, and some species may not cope with life in a warmer world (Peck 2011).

Most perciform fishes of the suborder Notothenioidei are endemic to the sub-zero marine waters of Antarctica (Bargelloni, Ritchie et al. 1994, Eastman 2000). The blood of almost all Notothenioidei contains antifreeze glycopeptides and has less hemoglobin, and they possess large muscle fibers which are adaptations to living in freezing waters compared with temperate ones (Bargelloni, Ritchie et al. 1994). Antarctic fish can acclimate to higher temperatures but take two to four times longer than temperate species (Bilyk and Devries 2011, Peck, Morley et al. 2014).

Is biodiversity changing in Antarctica and the Southern Ocean?

There are many examples of change in Antarctic biodiversity in terrestrial habitats due to climate change, visually dramatic examples of biological changes in response to climate warming include the colonization by macroscopic plants (largely mosses) of previously bare or newly exposed ground and the rapid expansion in extent and numbers of the only two higher plants present on the continent (Fowbert and Smith 1994, Convey and Smith 2006, Parnikoza et al. 2009, Convey 2011) but there has been much less research in the marine realm. As the climate changes, the frost-free periods in most mid and high latitude regions are lengthening and satellite data reveal a 10% decrease in snow cover and ice extent since the late 1960s (Hardy 2003). This has led to the successful colonization (after accidental introduction) of non-indigenous organisms such as the flightless midge *Eretmoptera murphyi* on Signy Island in the sub Antarctic (Hughes, Worland et al. 2012) and also accelerated desiccation of terrestrial organisms due to greater exposure times to damaging wavelengths of the radiation spectrum (Convey, Chown et al. 2014). In summer the integrated daily irradiance is as high, or even greater, in continental Antarctica than in many temperate areas (Convey, Chown et al. 2014). Terrestrial Antarctic biodiversity variation, although not particularly well documented aspects to be predominantly driven by abiotic factors such as nutrient and water availability. Estimates of the terrestrial biodiversity in the past can be attempted, if we know what the abiotic conditions were at that time (Convey 1996).

Historical proxies can be used to reconstruct a likely record of climate-induced long term biodiversity changes through geological time, which can be explained by distinct ecological and physiological links (Barnosky and Kraatz 2007). It is still difficult however, to interpret the consequences of environmental changes caused by climatic oscillations over time for marine organisms and community structure (Thatje, Hillenbrand et al. 2005). Antarctic coastal benthic communities are assumed to be especially sensitive to climate due to their high regional heterogeneity and uniqueness (Grange and Smith 2013). Thus, these are at relatively high risk under most climate change scenarios. They are

expected to significantly decrease or change their contribution to the overall Antarctic marine biological structure and functioning as well as ecosystem services (Grange and Smith 2013).

There has been a reduction in winter fast ice over the last few decades (Barnes and Souster 2011) and with predicted climate change scenarios (IPCC) this is likely to increase. For Antarctic benthic deep-sea organisms this could be beneficial, as less winter fast ice means a longer duration of the summer phytoplankton bloom and therefore longer periods of feeding which could mean more growth and increased biomass. However, for shallow water coastal benthic communities, increased glacial retreat and reduction in winter fast ice means an increase in the frequency of benthic ice scour (Barnes 2017a).

Aims and objectives of this thesis

The overall aim of this thesis was to study the biodiversity of Antarctic shallow water ecosystems and the organisms associated with shallow (<30m) hard substrata. This will provide detailed baseline data for future biodiversity comparisons. There are five main objectives, which investigate different aspects of biodiversity and biomass within a benthic marine environment, They are

1. To conduct seasonal comparisons of benthic biodiversity and biomass at 6, 12 and 20m on hard substrata at Rothera Point (Chapter 2). There have been no seasonal biodiversity/biomass comparisons carried out in Antarctica primarily due to winter logistical challenges and there are few benthic seasonal biodiversity comparisons worldwide. This study set out to determine if there is seasonal variation in the distribution and abundance of benthic marine invertebrates and the amount of organic carbon (biomass) on hard substrata. This will provide baseline data for future studies.
2. To undertake a temporal comparison of abundance and biomass of marine invertebrates on hard substrata at Rothera Point (Chapter 3). Barnes and Brockington (2003) reported a

survey completed in 1998 that assessed biodiversity, biomass and abundance at Cheshire Island, Rothera Point. Therefore this area was resampled to determine how shallow water biodiversity and biomass has changed in the 17 years between the surveys.

3. To undertake a seasonal comparison of metabolic rates in five species (those found to have a high biomass from the biodiversity surveys) of marine invertebrates found on hard substrata at Rothera Point (Chapter 4). The amount of organic carbon needed to be consumed to produce the energy required for growth, reproduction, maintenance and response to their environment was estimated. Animals use energy for all biological functions and it is therefore important to assess changes in metabolic rate and the need for organic carbon between summer and winter.
4. To carry out a pilot study of gut contents to investigate seasonal changes in diet in one of the common benthic filter feeders at Rothera Point, the holothurian *Heterocucumis steineni* (Chapter 5). This involved molecular barcoding to identify if the diversity of stomach contents changed between summer and winter. The molecular method was validated against morphological analysis using light microscopy and scanning electron microscopy of food items retrieved from the gut. If the prey items found using light microscopy and SEM were similar to that found by DNA barcoding, the method could be used to analyse the diets of other benthic marine invertebrates.
5. To synthesise the new insights provided by chapters 2-5 to produce a more holistic view of energy transfer within Antarctic nearshore benthic ecosystems (Chapter 6).

This PhD project contributes significantly to the study of biodiversity in Antarctic shallow water benthic ecosystems, including providing baseline data on rocky substratum meiofauna and energy flow by investigating sites near The British Antarctic Survey Base, Rothera Point, Adelaide Island.

Rothera Research Station

All of the research recorded here was carried out at Rothera Research Station (the main base for the British Antarctic Survey) situated on Adelaide Island, Western Antarctic Peninsula (67° 34' S, 68 ° 08' W, Figure 1.2a), Adelaide Island lies approximately 1860km south of the Falkland Islands and 1630km south east of Punta Arenas in Chile. Rothera lies roughly 120km inside the Antarctic Circle and consequently the sun remains below the horizon for a period of 4 - 5 weeks each year around mid winter (June 21st) and there is 24 hours of daylight for a similar period around mid summer.

The Western Antarctic Peninsula (WAP) (Figure 1.2a) was one of the fastest warming areas on the planet during the second half of the 20th century (Turner, Colwell et al. 2005). The climate of the Western Antarctic Peninsula was one of the most rapidly changing in the Southern Hemisphere, with a rise in atmospheric temperature of nearly 3°C between 1951 and 2000 (Vaughan, Marshall et al. 2003). Sea surface waters to the west of the WAP have warmed by more than 1 K since the 1950s, and the Circumpolar Deep Water (CDW) of the Antarctic Circumpolar Current has also warmed (Clarke, Murphy et al. 2007).

Sampling carried out by SCUBA

All marine invertebrate collections and biodiversity surveys detailed in this thesis were conducted through SCUBA diving. The length of the effective marine summer season with regard to water temperature varies from year to year depending on the length of winter fast ice. However, in this study SCUBA sampling was carried out all year round. The sea temperature reaches a maximum of around 1.5°C in the summer months between November and April. The minimum temperature does not fall below -1.8°C, the freezing point of the seawater. During the summer access to dive sites (sample sites) was by rigid inflatable boats (RIBs). During the winter access was with skidoo and sledge over the fast ice, where a hole was cut through the ice using a chain saw to gain access to the sea water beneath. Pressure, temperature, waves, and the need to carry air tanks dictate that sampling done by SCUBA has limitations due to time

restriction. For example, I was restricted to sampling to a maximum depth of 20m due to dive safety tables which meant 25 minutes at 20m, therefore any deeper and there just was not enough time to carry out the required sampling.

Typical challenges of sampling *in situ* in Antarctic waters relate to coping with low water temperatures and poor weather conditions at the surface, low visibility in summer months due to the intense phytoplankton bloom and brash ice conditions. Therefore to combat the sampling in low temperatures using SCUBA a 7mm dry suit was used, Arctic thermals, 5mm neoprene mitts and full face masks. The diving equipment used at Rothera Research Station is similar to that available to sports divers; except that the first stage has a glycol filled seal to prevent the low temperatures creating free flows (continuous flow of air when the diver is not inhaling). All diving is carried out under the UK Health and Safety Executive guidelines. Typically divers are restricted to 30m depth and therefore benthic surveys using diving are carried out in the near shore shallow regions.

Benthic biodiversity studies at Rothera

Marine science research has been carried out at Rothera since 1996 producing several long term data sets. The Rothera Time Series (RaTS) program is part of the long term monitoring of oceanographic science carried out at Rothera since 1997, providing a good understanding of interannual variability in a range of parameters. The RaTS programme includes profiling of physical properties of the water column, such as conductivity, temperature and depth using a conductivity, temperature and depth (CTD) instrument. Part of the RaTS sampling involves, depth stratified water column sampling for measurements of chlorophyll concentration, nutrients and isotopic tracers of ice melt. Linked to the sustained atmospheric and ice sustained observations at Rothera, RaTS represents one of the most comprehensive time series made anywhere in the Southern Ocean. RaTS data has led to advances in understanding of ocean changes in a region of rapid climate change (Henley, Tuerena et al. 2016). The measurements of chlorophyll, photosynthetic active radiation,

pH and oxygen influence near shore coastal biodiversity. Although temperatures are relatively stable in Antarctica, other factors including photoperiod and food availability vary markedly between seasons and years (Clarke 1988, Clarke, Meredith et al. 2008). Seasonality, productivity and ice scour are the three main factors affecting nearshore shallow water benthic biodiversity, therefore, the RaTS data should help to explain the biodiversity and biomass patterns found within this study.

Many Antarctic benthic marine invertebrates exhibit seasonal cycles related to variables monitored in the RaTS. For example, seasonality of food supply drives growth and reproduction, which in turn affects metabolic rate (Brockington, Clarke et al. 2001). In addition the RaTS program also carries out monthly collections of eight common Antarctic benthic marine invertebrates: the cushion star *Odontaster validus*, the brittle star *Ophionotus victoriae*, the limpet *Nacella concinna*, the sea cucumber *Heterocucumis steineni*, the terebellid worm *Thelepus Cincinnatus*, the nemertean worm *Parborlasia corrugatus*, the bivalve *Aequioidia eightsi* and the urchin *Sterechinus neumayeri* for reproductive studies; for example, these have increased our understanding of long term gametogenic ecology (Grange, Tyler et al. 2004, Grange, Tyler et al. 2007). Fast ice data extent and duration have been recorded as part of the RaTS since 1997 and annual measurements of ice scour frequency using marker grids have been carried out since 2003. Reduction in the duration of fast ice is strongly correlated with increased ice scour and mortality of benthos in the shallows (Barnes and Souster 2011). The effect of ice scour round Rothera Point has been studied intensively since 2000, looking at the influence of ice disturbance on near shore benthic communities (Brown, Fraser et al. 2004, Smale, Barnes et al. 2008).

There have been other short term projects researching shallow water marine biodiversity, ecology and physiology at Rothera. These include the seasonality of recruitment in Antarctic sessile marine benthos (Bowden 2005), the seasonal physiology and ecology of Antarctic marine benthic predators and scavengers (Obermüller, Morley et al. 2010), the *in situ* settlement of benthic fouling communities under future climate change scenarios (Ashton, Morley et al 2017) and the benthic biodiversity in Antarctic shallow water soft sediments (Vausse, Morley et al. in press). These projects have significantly contributed towards the general knowledge of marine biodiversity around Rothera Point and also provided important data underpinning the experiments and data interpretation within this thesis.

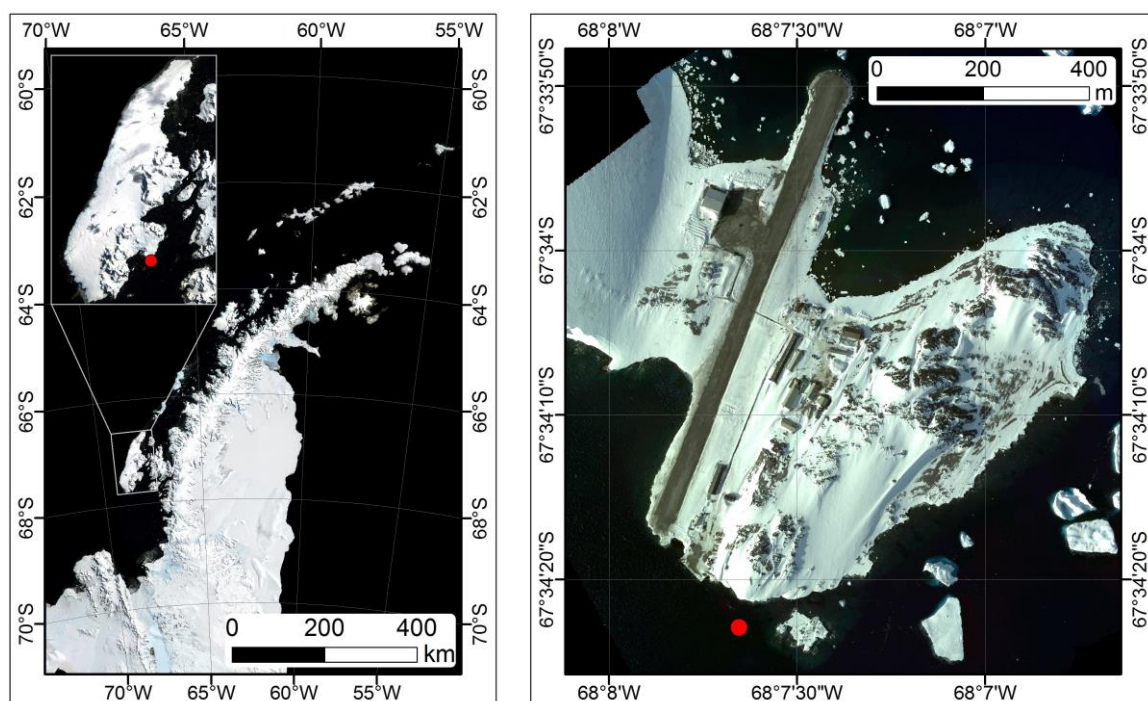


Figure 1.2 Western Antarctic Peninsula, showing Adelaide Island and Rothera Point showing the sample area in the south; Cheshire Island to South Cove

Some of the key scientific challenges for research in the Antarctic marine environment are how to improve estimates of biodiversity and to provide a better understanding of the ecology and physiology of the endemic fauna to enable accurate predictions of their responses to change. Only then can the biological information be used in conjunction with models of projected change in the region to predict the consequences for biodiversity (Griffiths 2010).

Chapter 2 - Biodiversity and biomass
of shallow water rocky substratum
communities: seasonal variation and
depth

Chapter 2 Biodiversity and biomass of shallow water rocky substratum communities: seasonal variation and depth

2.1 Introduction

Biodiversity describes the variety of life, including both the great variety of organisms and their varying behavior and interactions (Gaston and Spicer 2004). One of the overriding problems identified in ecology is that, in many key areas, biodiversity is to a large extent unquantified and consequently threats and losses cannot be measured let alone described (Hogg, Barnes et al. 2011). Thus it is essential to develop such baseline data, particularly in those regions where data are poor, or undergoing rapid change such as climate forcing. Baseline data could consist of what variety is present, in what number for a measured research effort level and how this varies with time (season and year)? This provides a measure of species richness (i.e. the pattern). Once established it is then possible to identify the processes which affect how ecosystems function. There are for example important questions such as, how much of each species is there in terms of its organic biomass, what are their ecological roles and importance to energy pathways in the system.

In order to protect biodiversity it is essential that we understand regional patterns and processes. According to Brey and Clarke (1993), average benthic biomass in the Antarctic is higher than that of temperate and sub tropical communities. One of the most widely recognized patterns in ecology is the increase in biodiversity that occurs from the poles to the tropics (Turner 2004). To make meaningful latitudinal comparisons a detailed biodiversity and biomass study across all phyla including a wide size range of each species representative of the benthic population is required. Barnes, Kaiser et al. (2009) found species richness of the South Orkney Islands to be dominated by marine species of which 83.3% were benthic. In Antarctica alone, there is ~5,500 km of ice-shelf-free shallow coastline (Smale, Barnes et al. 2008). Antarctica has around 2.6% of the worlds coastline but surprisingly > 8% of the worlds species in many

major groups of which the majority are endemic to the Antarctic (Arntz, Gutt et al. 1997). With 87% of marine glacier fronts currently in retreat on the Antarctic Peninsula (Cook, Fox et al. 2005, Scambos, Berthier et al. 2014) the Antarctic intertidal and shallow subtidal environment is undergoing dramatic environmental changes.

The documentation and management of changes in biodiversity requires accurate estimates of species richness and density (Brasier, Wiklund et al. 2016). Glacial retreat has the potential to expose ever-increasing areas of rocky or sedimentary shallow water habitats (Clark, Marzinelli et al. 2015). Antarctic biodiversity is much more extensive, ecologically diverse and biogeographically structured than previously thought. Life in the Antarctic and the Southern Ocean is surprisingly rich, and as much at risk from environmental change as it is elsewhere (Chown, Clarke et al. 2015). Accurate documentation of species richness and density is the primary step in understanding the patterns and controls of diversity levels, biogeography and functional ecology—all of which are fundamentally important to the management of marine ecosystems (Brasier, Wiklund et al. 2016).

There are currently 8,806 described species listed in the Register of Antarctic Marine Species. Gutt, Sirenko et al. (2004) predicted that, on the continental shelf alone, there could be as many as 17,000 species, implying that there are still a great many species yet to be described (Griffiths 2010). South of the Polar Front macrofaunal marine biodiversity is dominated by crustaceans, polychaetes, and molluscs (Arntz, Gutt et al. 1997, Clarke and Johnston 2003, Brandt and Hilbig 2004). Some of the earliest work on Antarctic marine biodiversity used the Mollusca, and more specifically the bivalves and gastropods as measures of biodiversity (Linse, Griffiths et al. 2006). Polychaete worms dominate in benthic marine communities including Antarctic waters where they can account for more than 70% of macrofauna (animals retained in a 300 μ m sieve) (Gambi, Castelli et al. 1997). Abundances in excess of 300 individuals 0.1m⁻² have been recorded (Sicinski 2011). Antarctic polychaetes show

cryptic diversity with as much as 50% of morphospecies targeted through the comparison of mitochondrial DNA sequences (Brasier, Wiklund et al. 2016). Other groups, especially the peracarid crustaceans, are exceptionally diverse in the Antarctic compared to other parts of the world (Clarke and Johnston 2003). More than one thousand endemic Antarctic peracarid species have been described and of these 531 are amphipods (De Broyer and Jazdzewski 1996). Isopods comprise 35% of all the peracarids in the Southern Ocean (Brandt, Brix et al. 2007).

Many groups of Antarctic marine animals and plants are thought to exhibit a high degree of endemism; for marine taxa this ranges from 35 to 90% of species (Arntz, Gutt et al. 1997). Echinoderms are generally conspicuous elements of Antarctic benthic communities, and they usually occur in fairly high densities and biomass in the deep sea (Gage and Tyler 1991, Brandt, Gooday et al. 2007). Echinoderms may comprise up to 55% of the benthic biomass at any given site in the Antarctic (Hétériér, David et al. 2008). In fact, echinoderms are well represented within existing Antarctic datasets, such as in the Scientific Committee on Antarctic Research Marine Biodiversity Information Network (SCAR-MarBIN), with high numbers of georeferenced records (Griffiths 2010). The Antarctic region is renowned for its isolated, unusual, diverse marine fauna. The holothurians are represented by 187 species (including 51 that are undescribed) recorded south of the Antarctic Polar Front which represents ~4% of the documented Antarctic marine biota, and ~10% of the world's holothuroid diversity (O'Loughlin, Paulay et al. 2011). Marine benthic macroalgae and microalgae were first recorded from Antarctic regions in the first half of the 19th century (Wulff, Iken et al. 2009).

The rocky shores along the Western Antarctic Peninsula support an extensive subtidal macroalgal assemblages, often covering 70% or more of the rocky seabed and with standing biomass stocks that rival those in temperate kelp forests (Quartino, Zaixso et al. 2005). The Antarctic pycnogonid fauna appears to be more diverse compared to that at lower latitudes; over 20% of the total global species known are found in

Antarctic or Sub-Antarctic waters, possibly being the most speciose area for pycnogonids in the world (Munilla and Soler Membrives 2008). The abundance and richness of bryozoans (8% of global species found in Antarctica) on temperate and polar continental shelves in the Southern Hemisphere coupled with their strong fossil record make them an ideal taxon for analyses of biogeography and biodiversity. Ascidians have a sessile adult lifestyle and direct development or a short-term planktonic stage of their larvae, which make them an excellent contrast group for biogeographical studies with the bryozoans because of the differences in dispersal potential, but there have been few studies on their distributional patterns (Primo and Vázquez 2007). Cnidarians are one of the most conspicuous taxonomic groups on the Antarctic continental shelf; they are a major component of the rich benthic communities, and have been described from several locations around Antarctica (Arntz, Brey et al. 1994, Gutt and Starman 1998).

Benthic marine biodiversity in Antarctica can be extremely patchy. Sponges can dominate Antarctic benthos with the numbers of Antarctic sponge species found in Southern seas being similar with patterns of species richness in temperate, tropical, and Arctic seas (McClintock, Amsler et al. 2005) or other phyla may be the most dominant. The phyla noted as most abundant and rich in the Antarctic often depends on the source used, as the information comes from researchers with expertise within a particular taxonomic group and location as benthic biodiversity can be extremely patchy.

Our knowledge of the biodiversity of the Southern Ocean is limited because of the relative inaccessibility of the region. Benthic sampling is largely restricted to the shelf; little is known about the fauna of the deep sea. The location of scientific bases heavily influences the described distribution pattern of sample and observation data, and the logistical supply routes are the focus of much of the at-sea and pelagic work (Griffiths 2010). Previous shallow water benthic biodiversity studies in the Southern Ocean include: soft sediment biodiversity research in Potter

Cove, King George Island (Pasotti, Manini et al. 2014), soft sediment biodiversity in King Edward Cove, South Georgia (Platt 1979), hard substratum biodiversity research at Rothera Point, Marguerite Bay (Barnes and Brockington 2003), Terra Nova Bay, Ross Sea (Gambi, Lorenti et al. 1994), Deception Island (Barnes, Linse et al. 2008) and South Orkney Islands (Barnes, Kaiser et al. 2009). Winter benthic studies in Antarctica are relatively scarce, owing mainly to difficulties in collecting during this period (Clarke 1996). There is therefore a great need for this benthic research into shallow water marine biodiversity to establish baseline information in the Antarctic if we are to understand the effect of climate change under predicted future scenarios.

Aims and objectives of this chapter

The aim of this chapter is to produce a benthic biodiversity baseline for shallow hard-rock near shore communities. This will be done by carrying out a benthic biodiversity survey.

(a) employing high levels of replication and adapting well-known benthic-sampling methods to enable detailed sampling of biodiversity and biomass measurements of all animals including encrusting species larger than 3mm in size.

(b) at different times of the year to incorporate possible seasonal differences. The coastal marine environment of Antarctica is characterized by extreme seasonality, a brief but intense summer period of open water and phytoplankton productivity alternating with winter sea ice, low light levels and reduced food availability for benthic suspension feeders. This will be the first seasonal benthic survey of hard rocky communities in the Antarctic.

(c) at different water depths. This study is the first near shore shallow water benthic survey to quantify benthic biodiversity and additionally to quantify organic biomass with depth in Antarctica. Once the biodiversity and biomass of the benthic community (this present chapter), the metabolic rates of these animals (Chapter 4) and their diets (Chapter 5)

are known we will be able to estimate if there is enough energy within the ecosystem to sustain them long term and under predicted future climate change scenarios.

Study site

The study site is adjacent to the British Antarctic Survey Research Station on Rothera Point at the southwest end of Adelaide Island, Western Antarctic Peninsula, 67°34'S 68°08'W. The site is directly adjacent to the ice cliffs at the southern end of the station, between Cheshire Island and the wharf (Figure 2.1). The topographic character of the area is a hard bedrock substratum with a steep gradient in places. The depth directly below the ice cliffs is 6m with a gradual slope down to 25m and then a steep drop off into 200+m. The annual sea temperature range varies between +1.5°C in the austral summer to -1.8°C in the austral winter (Clarke, Meredith et al. 2008) where the sea surface freezes to form fast ice for several months. Access to the study site was by rigid inflatable boat during the summer months and through holes cut in the sea ice in the winter months.

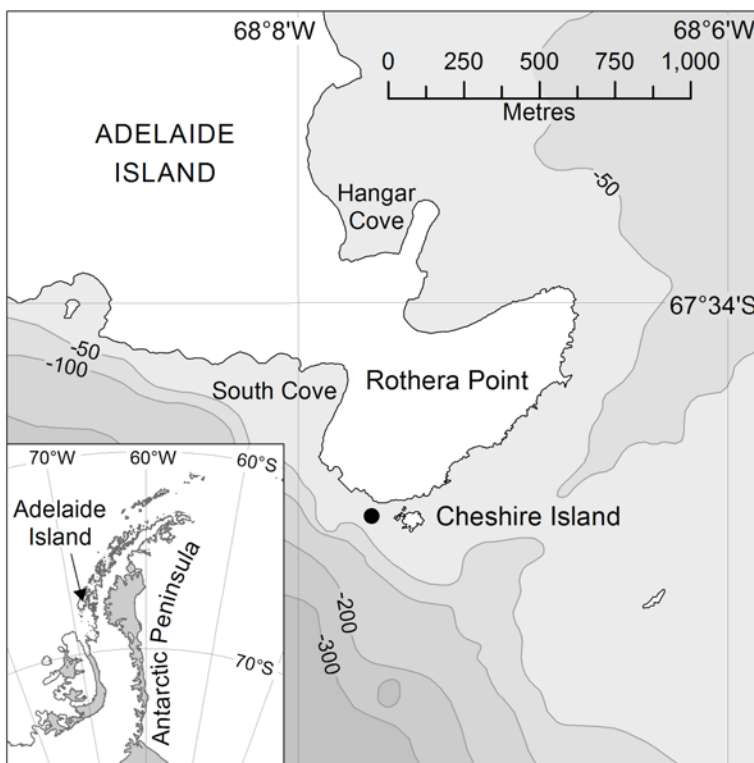


Figure 2.1 Map of the study site to the south of Rothera Point, Adelaide Island

2.2 Methods

The abundance and biomass of benthic taxa were estimated in January – March (austral summer) and June- October (austral winter) of 2015. Sampling of macrofauna (fauna collected by hand) and meiofauna (animals retained by a 3mm mesh) was carried out using SCUBA.

Preliminary inspection

Preliminary dives between Cheshire Island and the Rothera wharf were carried out to locate bedrock outcrops at 6m, 12m and 20m depth. Coordinates of suitable sampling sites were communicated to the dive boat using through water communication and were recorded on the GPS. (Figure 2.2)

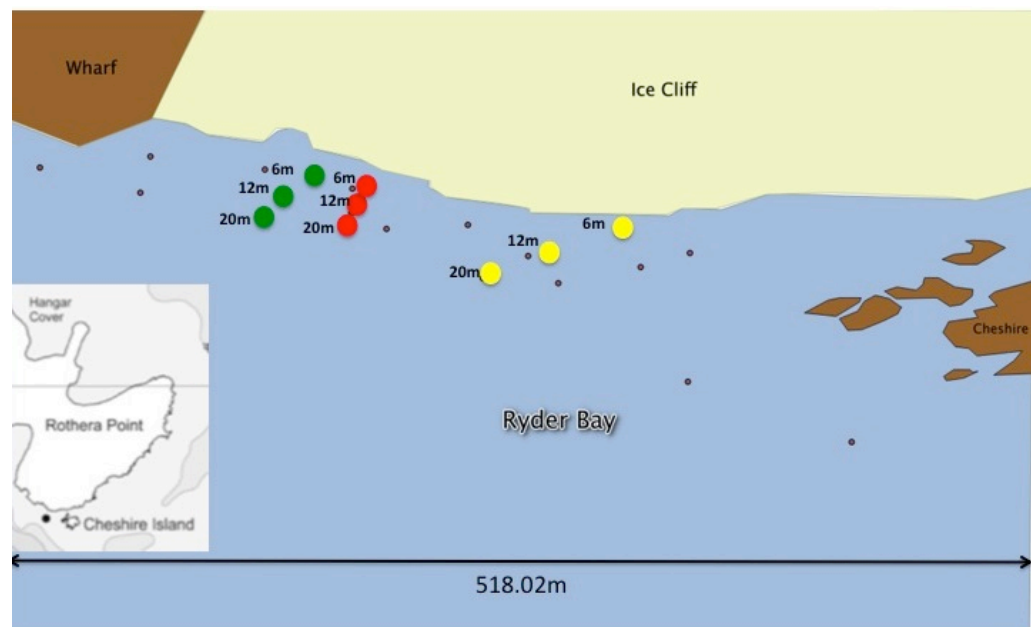


Figure 2.2 Depths and locations of identified hard substratum sites and the randomly selected transects, transect one (green), transect two (red) and transect three (yellow)

From these coordinates three transects covering the three depths were chosen at random using GIS mapping software.

Quadrat positioning

At the start of each sampling dive, a shotline was lowered from the dive boat at the target GPS coordinate. A 0.25m² quadrat was then placed as close to the shot as possible to remove human bias during placement.

During the winter months when the sea was frozen with fast ice, a dive hole was cut at the GPS location and the quadrat was placed in the dark before a light mounted on the frame was turned on, minimising human influence (Figure 2.3).

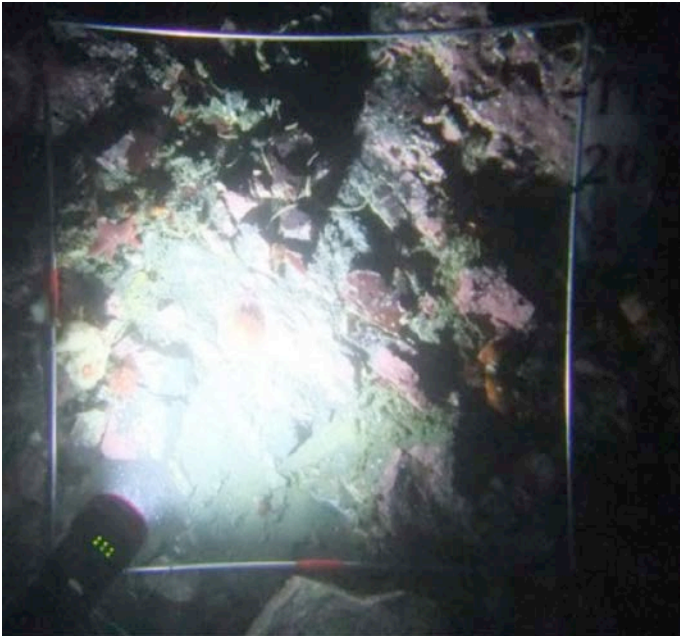


Figure 2.3 Picture showing under ice sampling in darkness with torch mounted on the side of the 0.25m² quadrat.

Experimental Sampling

Prior to commencing the sampling, a small white painted concrete block was placed next to the 0.25m² quadrat. The block prevented resampling of the same quadrat.

A photograph of the quadrat was taken before sampling commenced using a Fujifilm F300 EXR camera in underwater housing. Macrofauna was removed by hand and placed in 3mm mesh bags. Some meiofauna was also collected this way, typically attached to the larger animals. Rocks were typically collected at the end of sampling so that the meiofauna was not

disturbed prior to using the suction sampler. A custom designed and constructed suction sampler (fabricated at the British Antarctic Survey headquarters in Cambridge) was then used to collect all remaining meiofauna greater than 3mm (Figure 2.4).

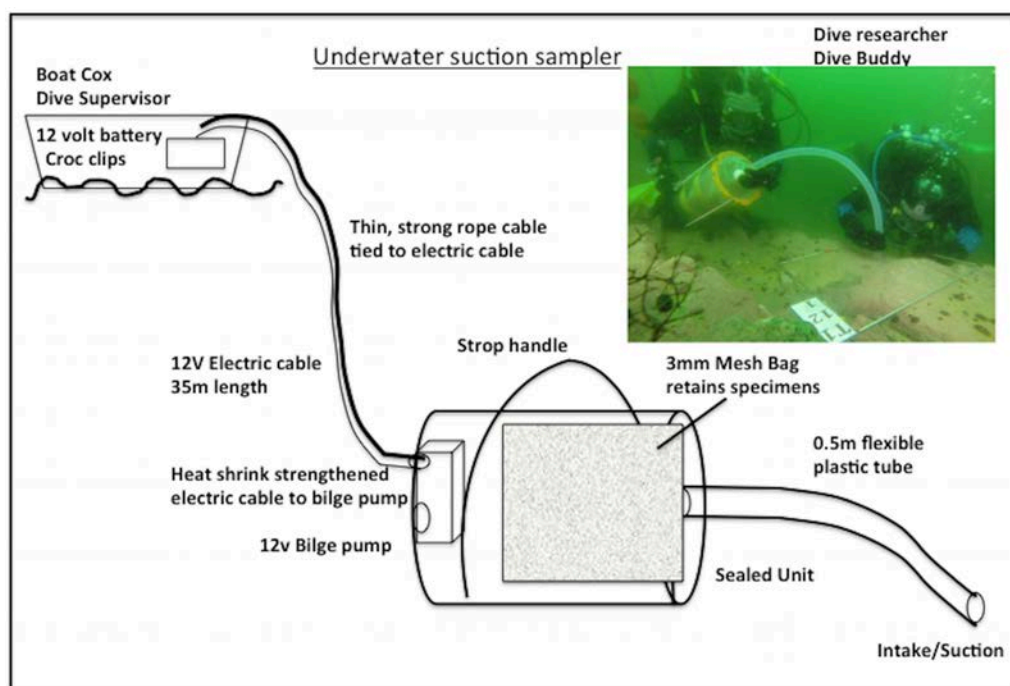


Figure 2.4 Suction sampler design, set up and operation (Souster and Yates design and manufacture).

The dive buddy held the suction sampler while the researcher sampled the entire 0.25m² quadrat area. Sampling continued until no remaining meiofauna (greater than 3mm) could be seen. Once sampling was complete, the pump was turned off at the surface, the plastic tube was removed and bungs placed in both the intake and outflow side of the suction sampler to prevent loss of water and potentially samples, on the surface. At the end of the sampling any loose rocks present that were within the quadrat on top of the bedrock were removed for assessment of encrusting fauna. A photograph of the final sampled quadrat was taken. On return to the dive boat, mesh bags containing macrofauna and rocks were placed in buckets containing ambient seawater to ensure animals remained submerged during transit. Buckets were taken to the research station. Upon arrival all animals were transferred to a controlled temperature aquarium facility on station (Flow through aquarium directly from Marguerite Bay) where they were held for subsequent examination.

Specimen sorting and identification

Macrofauna

Animals were identified using their morphological features and (ID guides: Polychaete worms Fauchald 1977, Antarctic Mollusca Dell 1990, The shelled Magellanic Mollusca Linse 2002, The Amphipoda Brueggeman 1998a, The Echinodermata Brueggeman 1998b) then separated by species into buckets containing ambient temperature sea water. Any animals that could not be identified to species level in the lab at Rothera Research Station were preserved (96% ethanol) for later identification by an expert or by barcoding.

Rocks

Rocks (if any) collected within the quadrat were placed in 5cm deep white trays containing ambient (aquarium) temperature sea water. Any non-encrusting animals were removed from the rock but retained in the tray. The rocks were labelled and allowed to dry for later analysis of encrusting fauna. Once dry, the encrusting bryozoan fauna on the rocks were identified using a high-powered microscope (magnification 5 – 480 and zoom 1 - 8) and identification guide (Hayward 1995). The area covered by each species of bryozoan was recorded using a cloth with 5mm x 5mm squares. The size of spirorbid worms was estimated by measuring the greatest diameter of the tube using Vernier calipers (Figure 2.5).

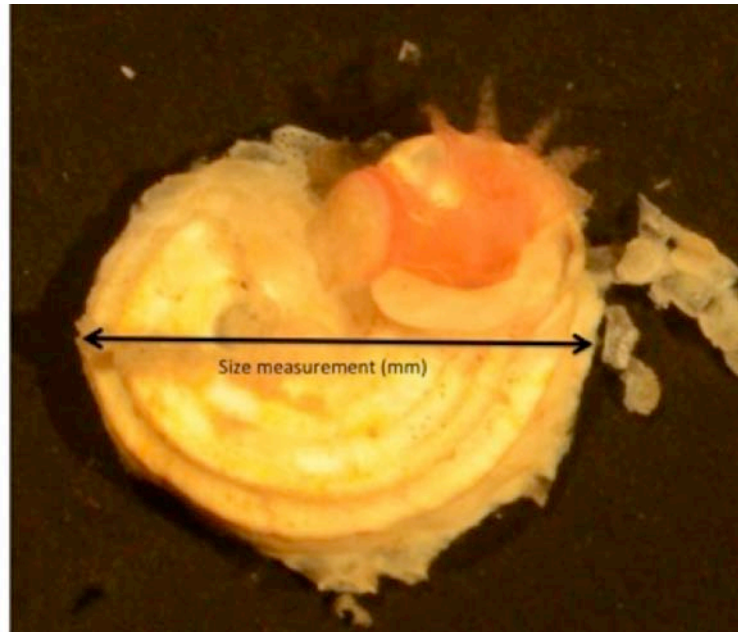


Figure 2.5 The spirorbid worm *Protolaeospira stalagmia* and the dimension (3mm) measured for this species

Meiofauna

The mesh bag from inside the suction sampler was removed and carefully emptied into trays of ambient temperature seawater in the station aquarium where the rocks had previously been inspected. Small containers (Diameter 58mm, Height 85mm, Figure 2.6) with mesh lids (2mm) that allowed water flow but retained the meiofauna were filled with ambient sea water and placed on top of snow collected from outside to maintain low sea water temperature while sorting the specimens. The animals from the suction sampler were then separated into different containers by taxonomic group (Figure 2.6). The first ten organisms of each unknown morphospecies were preserved (96% ethanol) for subsequent identification by an expert in that taxonomic group.



Figure 2.6. Meiofauna separated into different taxa, prior to identification and weighing. The top middle container shows the lids used to stop sampled animals from escaping during the analysis procedures

After sorting, the containers were kept submerged in tanks of free flowing ambient sea water until abundance and biomass measurements were made.

Analysis

Biomass measurements

All individuals of each species were counted before biomass measurements were obtained.

Macrofauna

Wet mass (WM) of macrofauna was obtained by rotating individuals three times on blue lab roll to blot them dry, then placing them in a pre-weighed aluminium boat on a balance (± 1 mg). After this, specimens were dried to constant mass at 60°C to assess dry mass (DM) and then incinerated at 475°C for 2-24 hours depending on the size of the specimen (approximate times required for combustion of organic carbon were ascertained during preliminary trials). Ash content was quantified as the mass post-incineration. Ash-free dry mass (AFDM) was calculated by subtracting ash content from dry mass (DM). AFDM was used as a measure of organic non-skeletal dry mass. For the abundant macrofauna, such as *Nacella concinna*, *Odontaster validus*, *Sterechinus neumayeri* and *Ophionotus victoriae*, AFDM mass was calculated for a minimum of thirty specimens from a wide size range representative of the general population. AFDM of further specimens of these species were estimated by interpolation from regression relationships between WM and AFDM (Figures 2.7 – 2.10). This was done because insufficient time was available to measure AFDM in all of the very common and abundant species. If the WM of a specimen was outside the WM used to calculate the regression, the organism was dried and ashed and the AFDM measurement obtained without regression. The same regression analysis was conducted for each of these species in the austral winter, as past studies in several species have provided evidence of a change in WM to AFDM relationship from summer to winter (Fraser, Clarke et al. 2002). The sea cucumber *Heterocucumis steineni* was one of the abundant macrofauna. However, the decision was made to ash all the specimens due to their large water content making the use of the WM to AFDM regression estimates unreliable.

AFDM to WM relationships for the common and abundant species in the survey were as follows:

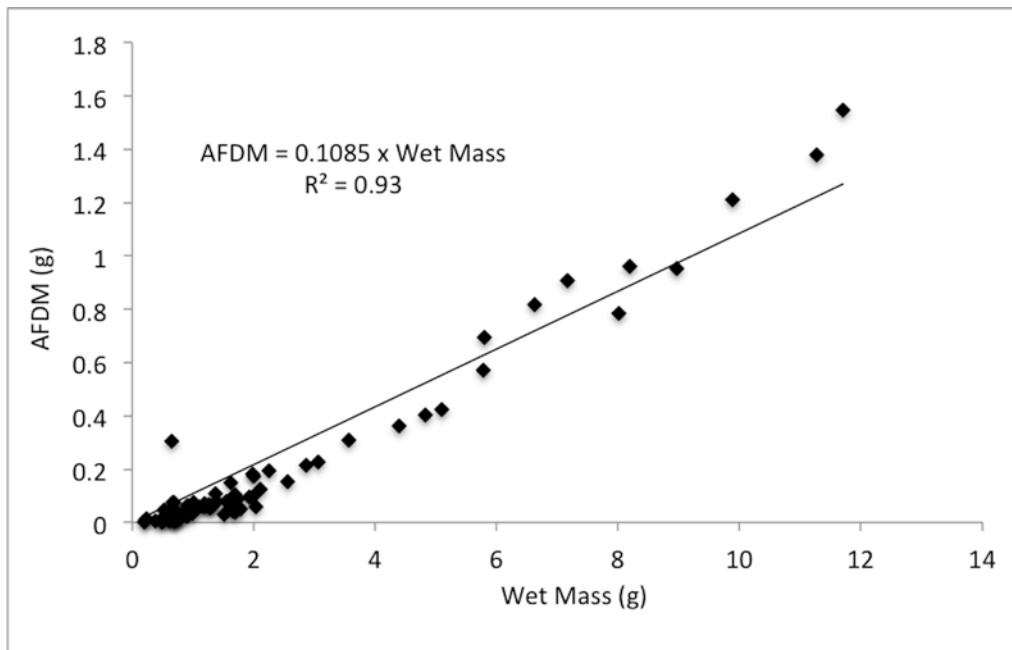


Figure 2.7. Austral summer ash-free dry mass (AFDM) expressed as a function of wet mass (WM) for the limpet *N. concinna*, ANOVA $F_{(1,73)} = 1744.4$ $P < 0.001$. Solid line represents line of best fit calculated using method of least squares.

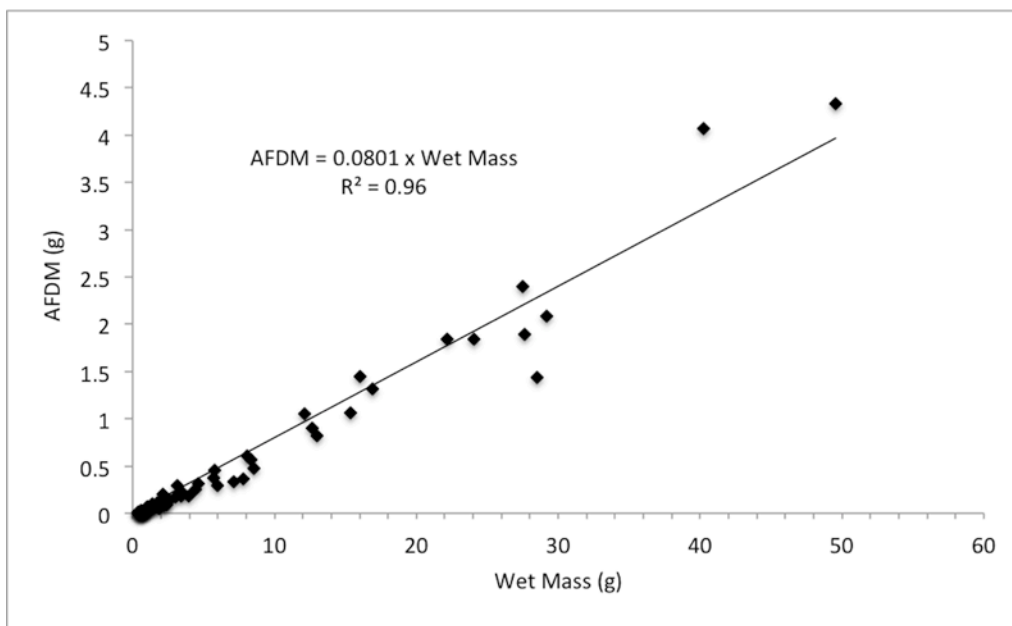


Figure 2.8. Austral summer ash-free dry mass (AFDM) expressed as a function of wet mass (WM) for the cushion star *O. validus*, ANOVA $F_{(1,78)} = 1964.3$, $P < 0.001$. Solid line represents line of best fit calculated using method of least squares.

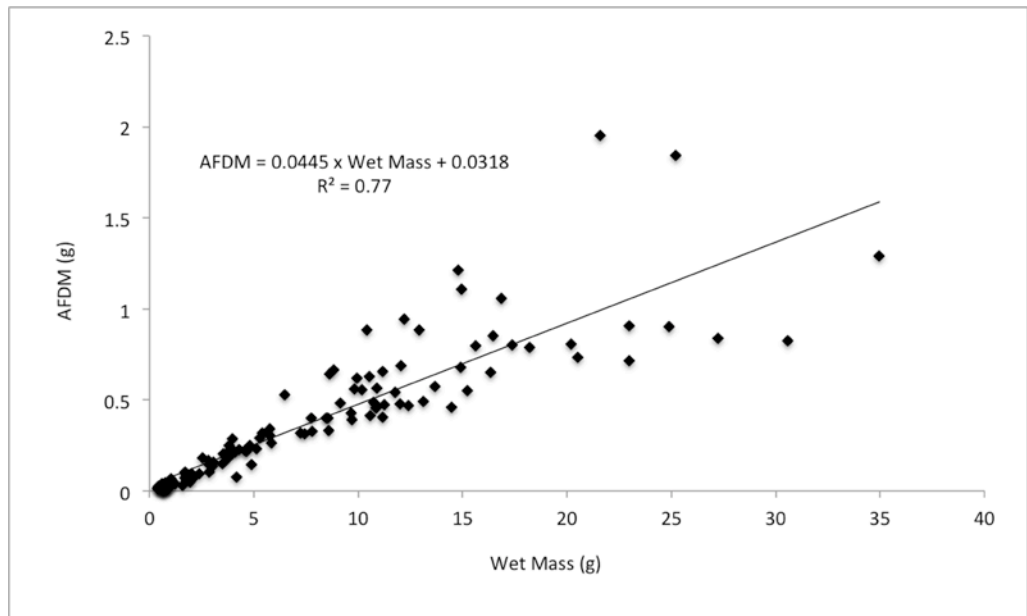


Figure 2.9. Austral summer ash-free dry mass (AFDM) expressed as a function of wet mass (WM) for the urchin *S. neumayeri*, ANOVA $F_{(1,113)} = 368.5$, $P < 0.001$. Solid line represents line of best fit calculated using method of least squares.

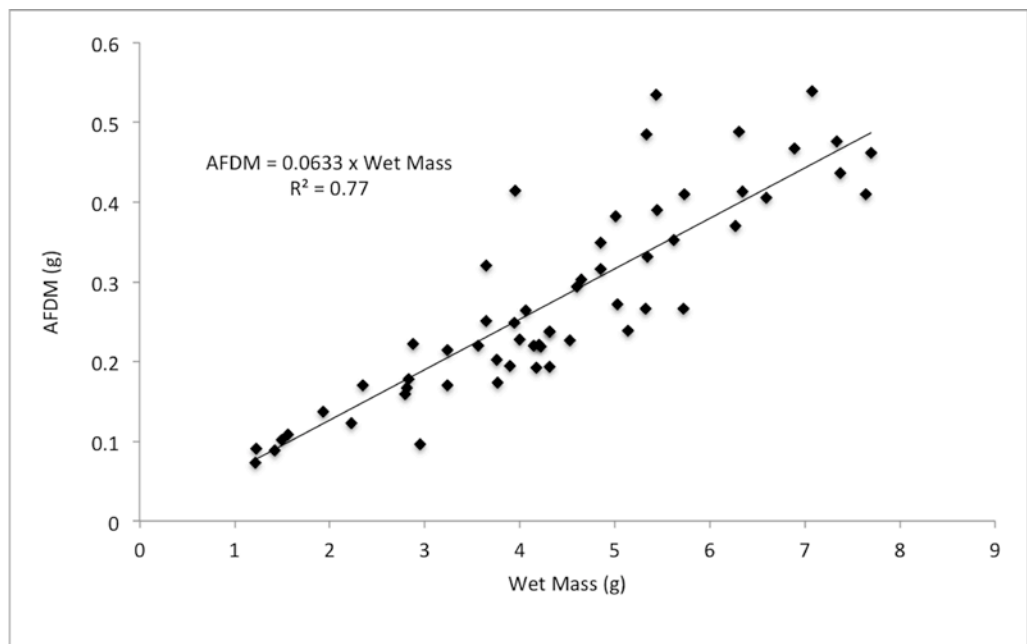


Figure 2.10. Austral summer ash-free dry mass (AFDM) expressed as a function of wet mass (WM) for the brittle star *O. victoriae*, ANOVA $F_{(1,57)} = 190.76$, $P < 0.0001$. Solid line represents line of best fit calculated using method of least squares.

Meiofauna

Each item of fauna was weighed (WM) in pre-weighed and pre-ashed aluminium boats (Figure 2.11) with excess water blotted using a small tip of blue roll. DM and AFDM were calculated using the same method as for macrofauna. All items of meiofauna, which were not preserved for identification, were dried and ashed for biomass measurements.



Figure 2.11 Species on aluminum boats about to be weighed to obtain dry mass (DM) and ash free dry mass (AFDM).

Rocks

Bryozoans

Due to the heterogeneity of the rock surfaces it was impossible to remove encrusting bryozoans without damaging them after they had been identified (Hayward 1995) and their surface area recorded. However, colonies were available from PVC panels deployed at 20m in both South Cove (Figure 1.2b) and Anchorage Island (within 4km from the site). Bespoke tools enabled sampling of bryozoans colonies covering five different surface areas (25mm², 50mm², 75mm², 100mm² and 150mm²) (Figure 2.12). The tools were circular with sharp metal circumference. A precise area from within a colony was sampled by twisting the extraction tool once in place. The tools were constructed on a metal lathe and the surface area of the extracted circle was calculated based on $A = \pi r^2$ (Table 2.1).

Area (mm ²)	Diameter (mm)
25	5.6
50	8.0
75	9.8
100	11.3
150	13.8

Table 2.1 Measurements for the extraction tools used to sample bryozoan colonies and remove a precise area of the colony



Figure 2.12. Picture of the bespoke extraction tools used to remove bryozoans from PVC panels to allow mass (AFDM) measurements to be made

A minimum of five replicates from each tool size area were taken where available. The live colony sample was placed in a pre-weighed aluminium boat and WM was measured using a Sartorius Genius ME215S balance ($\pm 0.015\text{mg}$). Specimens were then dried to constant mass at 60°C for DW before ash contents were obtained by incineration at 475°C for 2 hours and AFDM calculated as documented before. A regression was calculated for size (surface area) to AFDM for the common shallow water species *Fenestrulina rugula* (Figure 2.13).

There was no significant difference between the surface area: AFDM ratio for the bryozoans *F. rugula* and *Camptoplites bicornis* (T test $P = 0.29$ $N = 78$) therefore the data for both species were combined. These data were transformed logarithmically and a regression equation was calculated to allow the interpolation of the AFDM of a known surface area (Figure 2.14). If there was no significant difference between AFDM of an area for a different species of bryozoan, the same regression equation was used to estimate AFDM. For species of bryozoan where the AFDM to area relationship was significantly different, an average AFDM was calculated and then divided by the average of *F. rugula* to estimate the proportional difference in AFDM between the species (Figure 2.14). This correction factor was then applied to the value calculated from the *F. rugula*

regression for a given area. For example the bryozoan *Beania erecta* had a 3.8 fold larger AFDM than *F. rugula* when comparing the average AFDM for a given area. The value of AFDM for *Beania erecta* still fell within the variation (residuals) of the regression for *F. rugula* and AFDM calculated from the regression equation using the relationship of size to AFDM for *F. rugula* and *C. bicornis* was multiplied 3.8 (Figure 2.14).

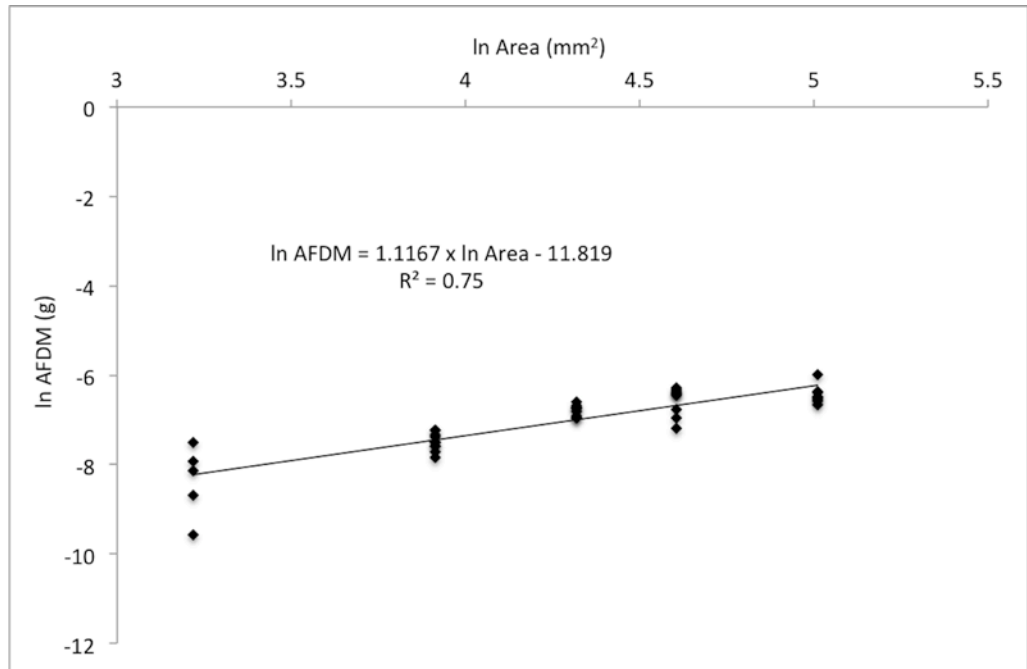


Figure 2.13. Logarithmically transformed AFDM to surface area relationship for the encrusting bryozoan *F. rugula*, ANOVA $F_{(1,40)} = 116.1$, $P < 0.001$.

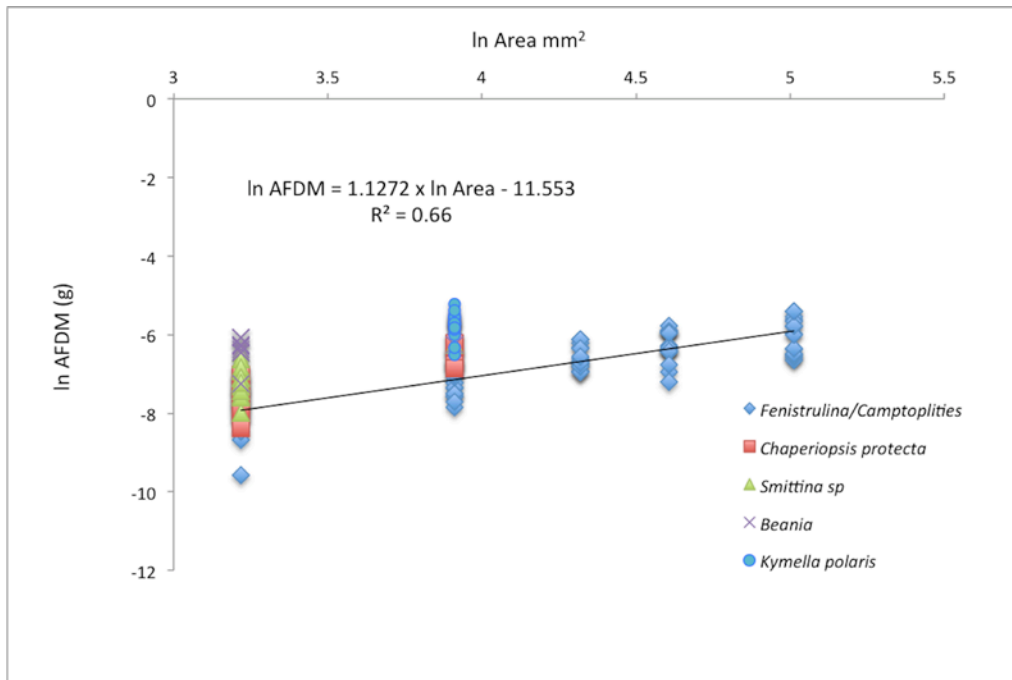


Figure 2.14 Surface area to AFDM data for other bryozoan species falling within the variability of the *F. rugula* relationship. This regression equation was also used for other species of shallow water encrusting bryozoans, ANOVA $F_{(1,78)} = 148.7$, $P < 0.001$. Line of best fit was calculated using the method of least squares.

Spirorbid worms

Spirorbid abundance and size (for all individuals > 3mm diameter) was recorded from all rocks found within the quadrats. To obtain the biomass (AFDM) estimates for the encrusting spirorbid worms, a collection of relatively flat rocks (chosen for ease of removal) containing a wide size range of the spirorbid population was made by SCUBA. Only worms, which were not encrusted by bryozoans, were used for mass measurements. There were two species of spirorbid worm; *Protolaeospira stalagmia* (anticlockwise spiral shell with ridge) and *Paralaeospira levinseni* (clockwise spiral shell smooth) found in the samples. Spirorbid worms were removed from the rock using a sharp scalpel and placed in pre-weighed aluminium boats to measure WM using a Sartorius Genius ME215S balance with $\pm 0.015\text{mg}$ accuracy. DW and AFDM were calculated as per meiofauna. A regression of the relationship between size and AFDM was obtained for each species. There was no significant relationship between AFDM and size (T Test $P > 0.05$ $N = 41$) therefore the

data were combined in one regression (Figure 2.15). The biomass of spirorbid worms within the quadrats was estimated using this regression equation (Figure 2.15).

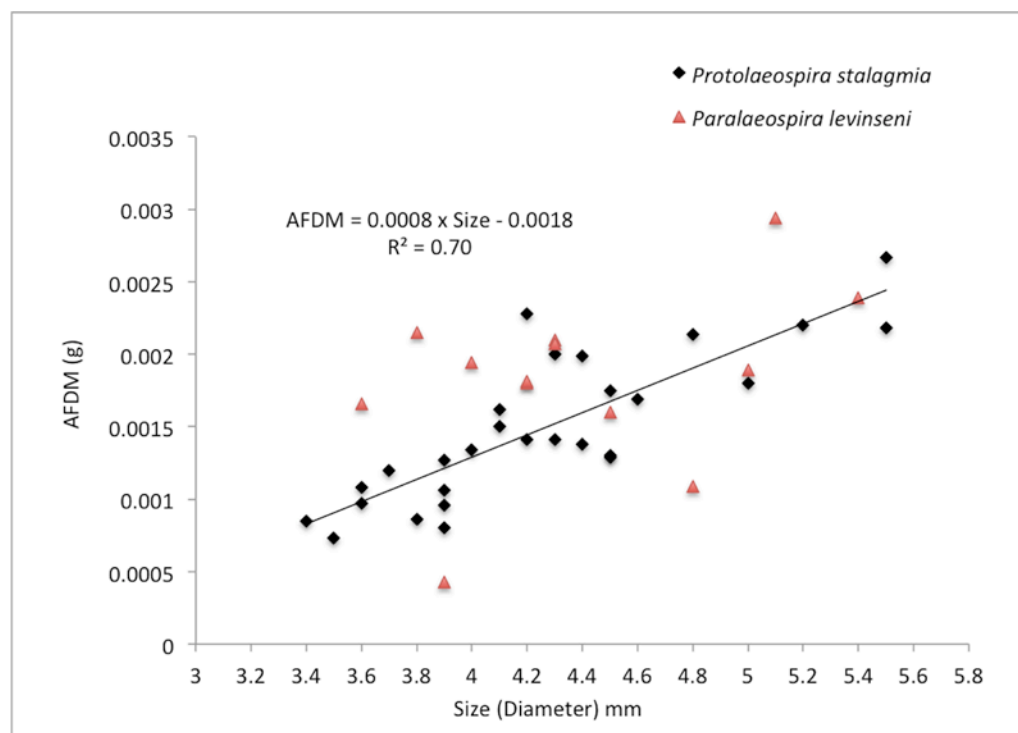


Figure 2.15 The relationship between size and AFDM for two species of spirorbid worms *Protolaeospira stalagmia* and *Paralaeospira levinseni*, ANOVA $F_{(1,40)} = 35.2$, $P < 0.001$. Line of best fit was calculated using the method of least squares.

Abundance and biomass values for each species within each 0.25m² quadrat were converted to values per m² for comparison with published data, future studies and for consistency.

Statistical analysis

Replicate samples from each depth in summer and winter were averaged to give faunal abundance, species richness and biomass m⁻². The data were collected using 0.25m² quadrats and then expressed as the mean number m⁻². Prior to analysis using a general linear ANOVA, the data were tested for normality using Anderson-Darling tests; if data were not normally distributed they were transformed using natural log. The data were also tested for heterogeneity of variance using Levene's test. Normal, homogeneous data were analysed using GLM (ANOVA), performed using

statistical analysis software (MINITAB) version 17 for Windows.

Shannon's Diversity Index (H') was calculated as a measure of diversity as it takes into account both species richness, and evenness of abundance among the species present. In essence it measures the probability that two individuals randomly selected from an area will belong to the same species. The values (H') for Shannon's Index are generally between 1.5 and 3.5 in most ecological studies, and the index is rarely greater than 4. The Shannon Index increases as both the richness and the evenness of the community increase (Magurran 2004)

Multivariate analyses, using routines within the PRIMER statistical package (PRIMER -e Ltd, Plymouth, U.K), were used to examine relationships between seasons and depths for all taxa. Bray Curtis similarity matrices were produced based on the abundance and biomass data of the meio and macro fauna, which were pre-treated with fourth root transformations to downweight the influence of highly abundant or large taxa. Non-metric multi-dimensional scaling (nMDS) ordinations were used to visualise relationships between samples. The clustering within nMDS was tested statistically using SIMPROF groups with 999 permutations, and any difference between depths and season was tested using a two way crossed analysis of similarities (ANOSIM). SIMPER analysis was then used to test which species were responsible for any differences between depths and seasons. The relationships with seasonal patterns were also tested using the RELATE correlation procedure. The technique tests for correlations between matrices of Bray-Curtis similarities generated from the sample data in summer and the Bray-Curtis similarities generated from the sample data in winter, ρ will be zero if there is no relationship between the similarity matrices, whereas ρ closer to 1 indicates that there is a relationship between the similarity matrices within the two biotic arrays.

2.3 Results

Figure 2.16 presents an overall summary of the sampling sites, transects and depths with pie charts representing the percentage contribution of selected phyla. A total of 148 taxa, representing 15 phyla and 19 classes were recorded including 97 taxa identified to morphospecies level (Table 1). The most speciose phylum was the Arthropoda with 30 different taxa, followed by Mollusca with 26, and Bryozoa with 22 species. None of the individual samples contained the full range of taxa recorded in the overall survey, the highest number of taxa per m² was 59 species recorded at 20m depth. During the entire study, 57 sample dives were carried out including 30 samples in the austral summer and 27 in the austral winter. The samples collected during a dive took an average of three days to process in the laboratory, including identification and making biomass (AFDM) measurements.

Subtidal biomass increased with depth and varied with season. The lowest biomass measured was in winter at 6m depth (17.44 g m⁻² SE = 2.32) and the highest biomass was at 20m depth in summer (173.68 g m⁻² SE = 86.08). The three phyla contributing the greatest amount of organic carbon across all depths were Mollusca, Echinodermata and Annelida (Figure 2.29). The lowest biomass recorded was from a single quadrat at transect one, 6m depth in winter (4.83 g m⁻²) and the largest biomass recorded in a single quadrat was at transect three, 20m depth in summer (937.50 g m⁻², with the holothurian *H. steineni* contributing 630.85 g m⁻²).

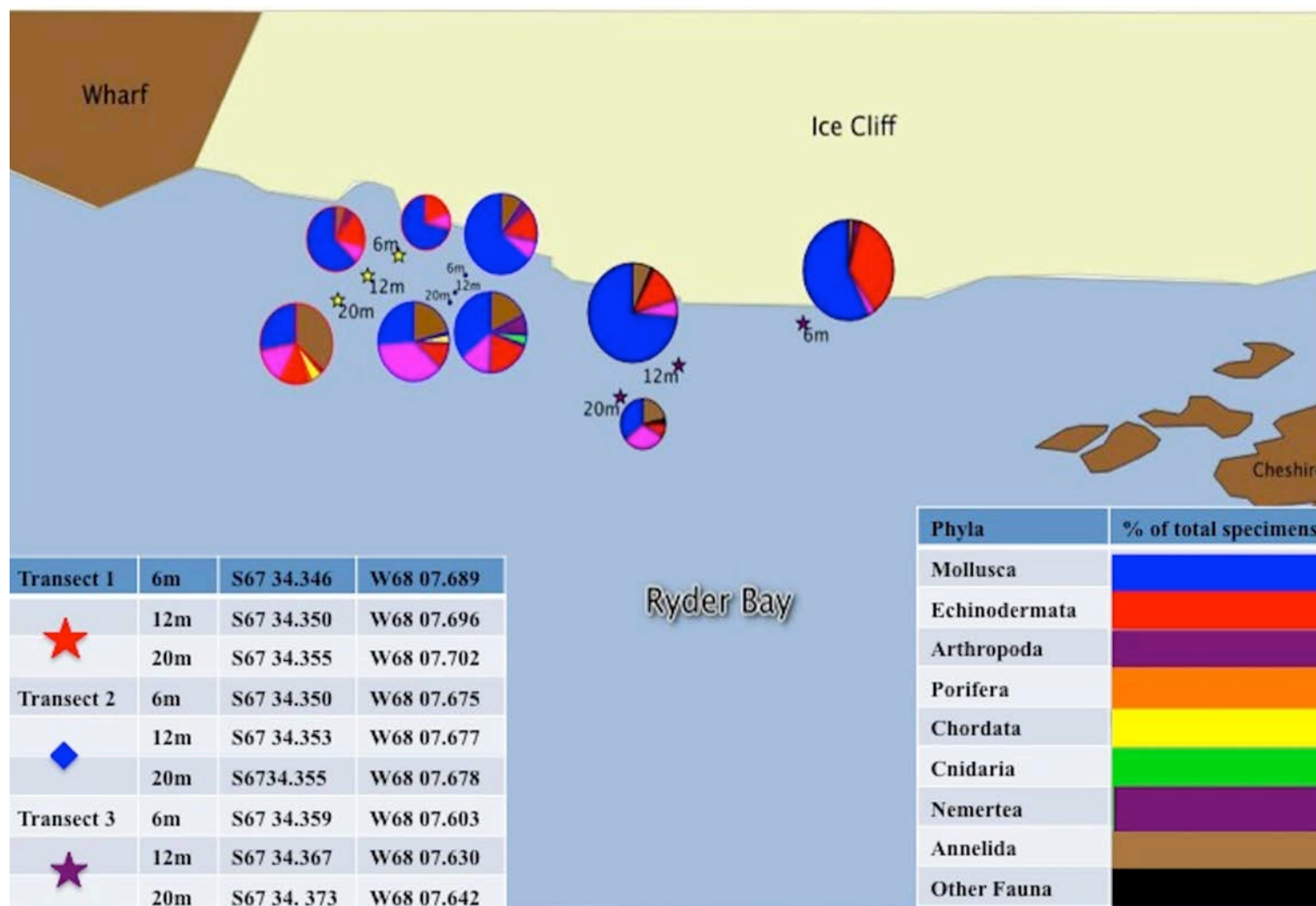


Figure 2.16. QGIS map showing locations of the three hard rock biodiversity transects and a pie chart summary of the proportion (%) of different phyla. Other organisms include: Brachiopoda, Ochrophyta, Rhodophyta, Platyhelminthes, Sipuncula and Nematoda which had few species. Bryozoa are excluded from this diagram as this phylum was measured by area and not number of individuals.

Taxon Identification

For some taxa, identification to species level was not possible especially for meiofauna and Annelida. Species that could not immediately be identified were preserved in 96% ethanol with a code and later identified by a taxonomic expert in that field (Table 2.2). Mollusca, were identified by Dr Katrin Linse of the British Antarctic Survey. Dr Michael Schroedl and Enrico Schwabe of Zoologische Staatssammlung, München assisted Dr Katrin Linse with nudibranch and chiton identification, respectively. Professor Lloyd Peck confirmed all my species of Brachiopoda were *Liothyrella uva*. Camille Moreau of The Université Libre de Bruxelles assisted with the identification of Asteroidea and Ophiuroidea while Melanie Mackenzie of Victoria Museum Australia with the identification of holothuroidea. Of the Arthropoda phylum Dr Anna Jazdzewska from The University of Lodz identified the amphipod species, Dr Stefanie Kaiser from Senckenburg the Isopoda species and Dr Jana Doemel from Universitat Duisberg-Essen the pycnogonid species. Dr Claire Goodwin from The Huntsman Marine Science Center Canada identified all the porifera samples. Professor Frithjoff Kuepper from The University of Aberdeen identified the Ochrophyta and Rhodophyta. Dr Megan Schwartz from The University of Washington identified the nemertea and also barcoded them for the Genbank database. Of the phyla Chordata and Cnidaria, the most abundant species were identified, although some of the smaller ones were not and therefore are recorded as sample numbers. There are few Antarctic annelid experts and those contacted were unable to help with identifications. As it was not possible to identify specimens from this group to species level on morphological criteria, 18S barcode genetic identification of the annelid species present was made to the lowest reliable taxonomic unit, family. The barcoding allowed us to distinguish different species, but as we could not identify the species, they were labeled as unidentified taxonomic units (UTU). The bryozoans were identified under high power magnification (Nikon SMZ800 with magnification dependent on different species) with assistance from Dr Gail Ashton and Dr David Barnes.

Phylum	Class	Order	Family	Species
Mollusca				
Dr Katrin Linse	Gastropoda		Nacellidae	<i>Nacella concinna</i>
			Lepitidae	<i>Iothia</i> sp
			Eatoniellidae	<i>Eatoniella caliginosa</i>
				<i>Eatoniella cf. glacialis</i>
				<i>Eatoniella</i> sp
		Vetigastropoda	Calliostomatidae	<i>Margarella antarctica</i>
			Rissoidae	<i>Onoba grisea</i>
				<i>Onoba cf. turqueti</i>
			Littorinidae	<i>Laevilitorina antarctica</i>
			Muricidae	<i>Trophon cf. minutus</i>
			Naticidae	Sp
Dr Michael Schroedl and Dr Katrin Linse		Nudibranchia	Charcotiidae	<i>Charcotia granulosa</i>
				<i>Cuthona cf. modesta</i>
				<i>Pseudotritonia quadrangularis</i>
Dr Katrin Linse	Bivalvia	Arcida	Philobryidae	<i>Philobrya wandelensis</i>
				<i>Philobrya sublaevis</i>
				<i>Adacnarca nitens</i>
			Cyamiidae	<i>Cyamiomactra laminifera</i>
		Limida	Limidae	<i>Limatula ovalis</i>
		Nuculanida	Yoldiidae	<i>Aequiyoldia eightsi</i>
				<i>Altenaeum charcoti</i>
				<i>Melanella</i> sp
Enrico Schwabe	Polyplacophora	Chitonida	Ischnochitonidae	<i>Tonicina zschau</i>
			Hemiarthridae	<i>Hemiarthrum setulosum</i>
		Lepidopleurida	Leptochitonidae	<i>Leptochiton kerguelensis</i>
Brachiopoda Prof Lloyd Peck	Rhynchonelliformea	Terebratulida	Terebratulidae	<i>Liothyrella uva</i>
Echinodermata				
Terri Souster and Camille Moreau	Asteroidea	Valvitida	Odontasteridae	<i>Odontaster validus</i>
				<i>Odontaster roseus</i>
				<i>Odontaster</i> sp
		Forcipulatida	Asteriidae	<i>Cryptasterias turqueti</i>
				<i>Diplasterias brucei</i>
				<i>Cuenotaster involutus</i>
				<i>Pisaster ochraceus</i>
				<i>Lysasterias</i> sp
	Echinoidea	Camarodonta	Echinidae	<i>Sterechinus neumayeri</i>
	Ophiuroidea	Ophiurida	Ophiuridae	<i>Ophionotus victoriae</i>
				<i>Ophiura crassa</i>
			Ophiodermatina	<i>Ophiopsammus maculata</i>
Melanie Mackenzie	Holothuroidea	Dendrochirotida	Cucumariidae	<i>Heterocucumis steineni</i>
				<i>Echinopsolus charcoti</i>
				<i>Echinopsolus acanthocola</i>
				<i>Cucumaria</i> sp

		Aspidochirotida	Synallactidae	<i>Pseudostichopus peripitus</i>
Arthropoda				
Dr Anna Jazdzewska	Malacostraca	Amphipoda	Pontogeneiidae	<i>Prostebbingia brevi/longicornis</i>
				<i>Schraderia gracilis</i>
				<i>Prostebbingia gracilis</i>
				<i>Eurymera monticulosa</i>
				<i>Paramoera</i> sp
				<i>Gondogeneia</i> sp
			Eusiridae	<i>Rhachotropis antarctica</i>
			Phoxocephalidae	sp
				<i>Heterophoxus videns</i>
			Lysianassoidea	<i>Cherimedes femoratus</i>
			Exoedicerotidae	<i>Parhalimedes turqueti</i>
				<i>Methalimedes nordenskjoldi</i>
			Calliopiidae	<i>Oradarea</i> sp
				<i>Oradarea cf. walker</i>
			Oedicerotidae	<i>Monoculodes scabriculosus</i>
			Liljeborgiidae	<i>Liljeborgia</i> sp.
			Hadziidae	<i>Paraceradocus miersi</i>
		Cumacea		sp1
Dr Stefanie Kaiser		Isopoda	Janiridae	<i>Iathrippa cf.sarsi</i>
			Munnidae	<i>Munna antarctica</i>
			Natatolana	sp1
			Janiridae	sp1
			Gnathiidae	sp1
			Sphaeromatidae	<i>Cymodocella tubicauda</i>
Dr Jana Doemel	Pycnogonida	Pantopoda	Callipallenidae	<i>Austropallene</i> sp
			Ammotheidae	<i>Achelia</i> sp
			Austrodecidae	<i>Pantopipetta</i> sp
			Nymphonidae	<i>Pentanymphe antarcticum</i>
			Endeis	<i>Australis</i> sp
			Pycnogonidae	<i>Pycnogonum</i> sp
Porifera	Demospongiae	Dendroceratida	Darwinellidae	<i>Dendrilla antarctica</i>
Dr Claire Goodwin		Haplosclerida	Chalinidae	<i>Haliclona</i> sp1
				<i>Haliclona</i> sp2
			Niphathidae	<i>Pachychalina</i> sp1
		Poecilosclerida	Acarnidae	<i>Iophon hesperidesi</i> rios
				<i>Megaciella</i> sp
			Myxillidae	<i>Myxilla ectyomyxilla</i>
		Polymastiida	Polymastiidae	<i>Sphaerotylus antarcticus</i>
		Suberitida	Suberitidae	<i>Suberites topsenti</i>
				<i>Protosuberites</i> sp1
Ochrophyta				
Prof Frithjof Kuepper	Phaeophyceae	Desmarestiales	Desmarestiaceae	<i>Desmarestia menziesii</i>
		Ectocarpales	Adenocystaceae	<i>Adenocystis utricularis</i>
Rhodophyta				
Prof Frithjof Kuepper	Florideophyceae	Gigartinales	Kallymeniaceae	<i>Kallymenia antarctica</i>
		Rhodymeniales	Rhodymeniaceae	<i>Rhodymenia subantarctica</i>

			Fryeellaceae	<i>Hymenocladopsis crustigena</i>
		Palmariales	Palmaraceae	<i>Palmaria decipiens</i>
	Florideophyceae	Plocamiales	Plocamiaceae	<i>Plocamium</i> sp
Chordata	Ascideacea	Stolidobranchia	Styelidea	<i>Cnemidocarpa verrucosa</i>
				ASC01
				ASC02
				ASC03
Cnidaria	Anthozoa	Alcyonacea	Alcyoniidae	<i>Alcyonium antarcticum</i>
				CNI01
				CNI02
				CNI03
Nemertea				
Dr Megan Schwartz	Anopla		Lineidae	<i>Parborlasia corrugatus</i>
				<i>Cerebratulus cf longifissus</i>
			Valenciniidae	<i>Baseodiscus cf antarcticus</i>
Annelida				
Dr Melody Clark	Polychaeta		Capitellidae	UTU1
			Hesionidae	sp
			Nereididae	UTU1
				UTU2
			Opheliidae	UTU1
			Orbiniidae	UTU1
				UTU2
			Polynoidae	UTU1
				UTU2
			Sabellidae	sp
			Syllidae	UTU1
				UTU2
		Terebellida	Terebellidae	<i>Thelepus Cincinnatus</i>
				UTU1
				UTU2
				UTU4
		Phyllodocida	Nephtyidae	<i>Aglaophamus trissophyllus</i>
		Sabellida	Serpulidae	<i>Protolaeospira stalagmia</i>
				<i>Paralaeospira levinseni</i>
Platyhelminthes				
Juliana Bahia (Dr Michael Schroedl PhD student)	Rhabditophora	Polycladida	Acotylea (sub order)	UTU1
			Prosthiostomidae	UTU1
			Stylochoididae	<i>Stylochoides albus</i>
Sipuncula	Sipunculidea			SP1
Nematoda				UTU2
Bryozoa				
Terri Souster	Gymnolaemata	Cheilostomatida	Buffonellodidae	<i>Aimulosia antarctica</i>
with assistance from				<i>Arachnopusia inchoata</i>
Dr David Barnes			Beaniidae	<i>Beania costata</i>
Dr Gail Ashton				<i>Beania erecta</i>

			Bugulidae	<i>Camptoplites bicornis</i>
			Hippothoidae	<i>Celleporella antarctica</i>
			Chaperidae	<i>Chaperiopsis protecta</i>
			Calloporidae	<i>Ellisina antarctica</i>
			Exochellidae	<i>Escharoides tridens</i>
			Microporellidae	<i>Fenestulina exigua</i>
				<i>Fenestulina rugula</i>
			Cribrilinae	<i>Figularia discors</i>
				<i>Filaguria spatulata</i>
				<i>Hippadanella inerma</i>
				<i>Himantazoum antarcticum</i>
				<i>Kymella polaris</i>
			Microporellidae	<i>Micropora notialis</i>
				<i>Micropora sp 2</i>
			Smittinidae	<i>Smittina sp</i>
			Lacernidae	<i>Toretocheilum absidatum</i>
	Stenolaemata	Cyclostomatida	Tubulipora	<i>Tubulipora sp</i>

Table 2.2. Full taxon list showing the phylum, class, order, family and species found on the shallow bed rock south of Rothera Point. The taxonomic experts that did the identifications are given below each major taxon

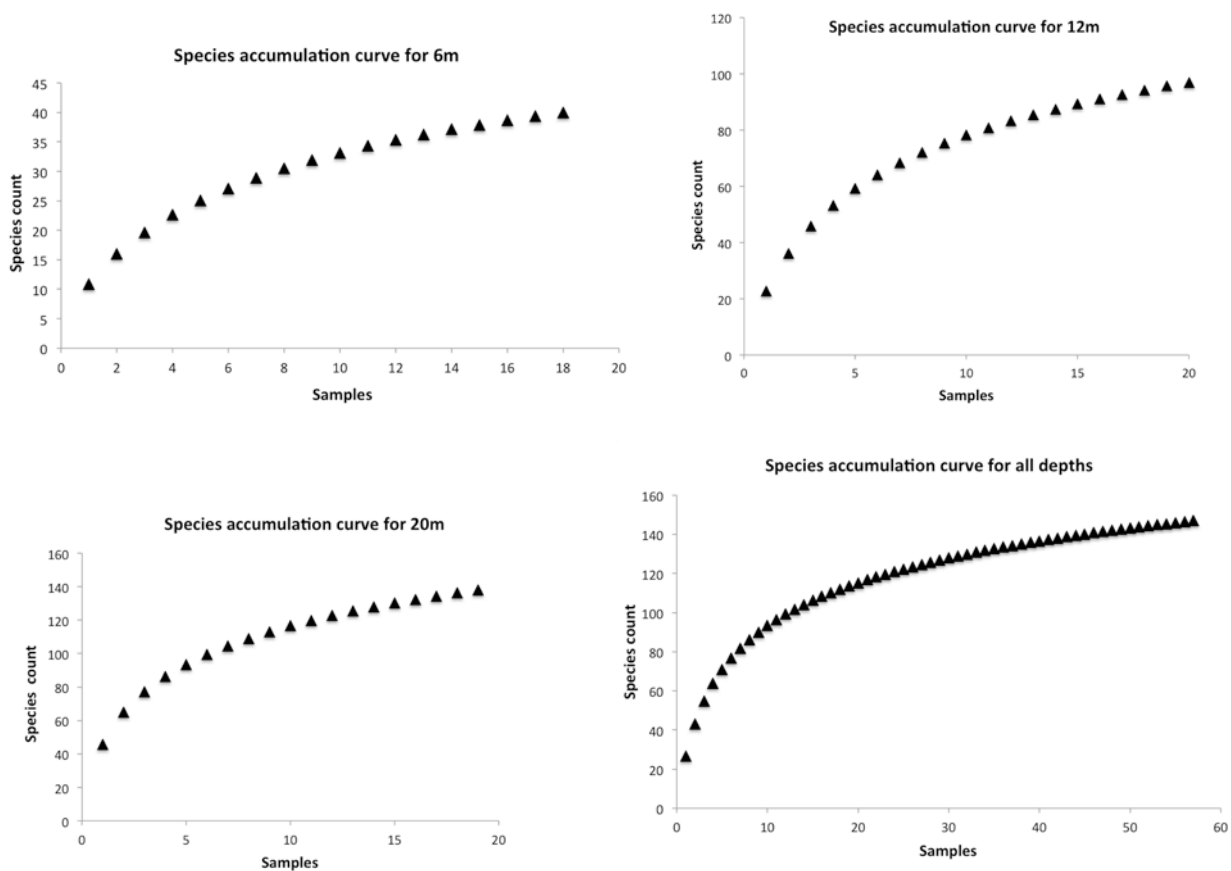


Figure 2.17. Species accumulation curves by depths (6m, 12m and 20m) and across all depths in both summer and winter as there was no significant difference between seasons.

Species accumulation curves for each depth indicate that the majority of species found at that depth on hard rocky sub strata were probably sampled (Figure 2.17). However the gradients of the curves for each depth: 6m, 12m, 20m and even the overall summary for all three depths combined are still rising slightly after 18, 20, 19 and 57 samples respectively suggesting that more species remain unsampled at these depths.

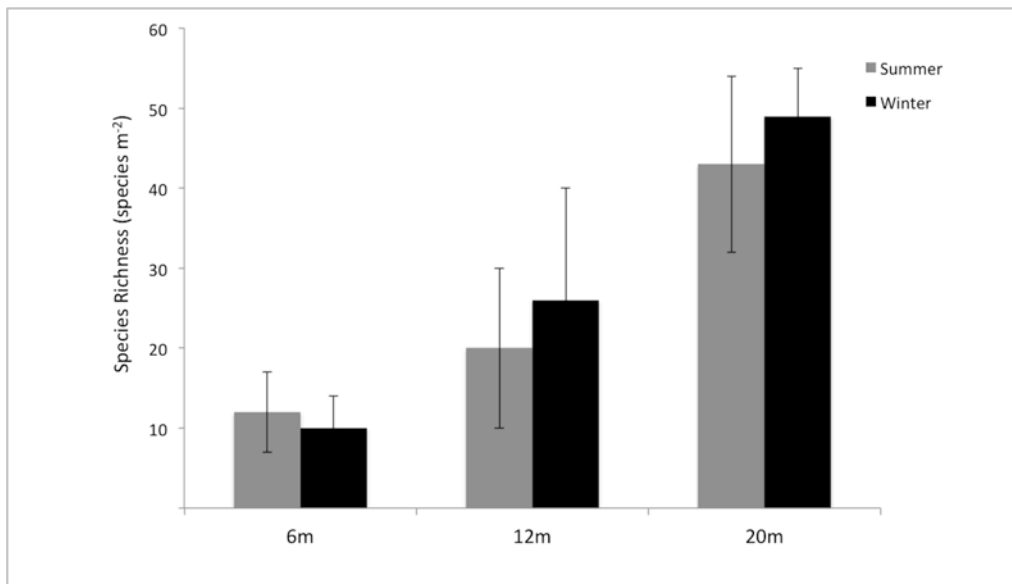


Figure 2.18. Mean species richness at 6m,12m and 20m in the austral summer of 2015 (grey bars) and the austral winter (black bars) in 2015 +/- SD.

Species richness was not significantly different between, summer and winter seasons in 2015 (GLM $F_{(1,56)} = 1.55$, $P = 0.22$ Figure 2.18). There was also no significant species interaction between season and depth (GLM $F_{(2,56)} = 0.91$, $P = 0.41$). However species richness was significantly different between depths (GLM $F_{(2,56)} = 69.64$, $P < 0.001$). The highest mean species richness recorded, 52 species m⁻² was in the winter at 20m. The lowest species richness, 10 species m⁻² was recorded in winter at 6m.

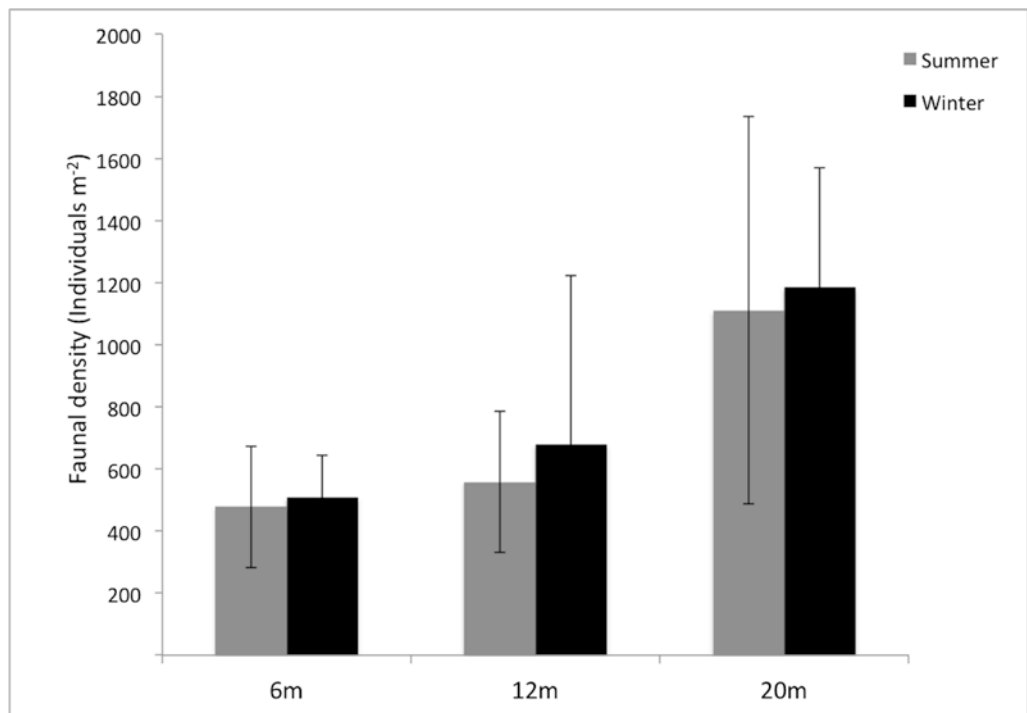


Figure 2.19. Mean density at 6m, 12m and 20m in the austral summer 2015 (grey bars) and the austral winter 2015 (black bars) +/- SD.

There was no significant difference between mean densities in either season (GLM on natural log transformed data $F_{(1,56)} = 0.73$, $P = 0.396$ Figure 2.19). There was however, a significant difference between depths (GLM on natural log transformed data $F_{(2,56)} = 16.28$, $P < 0.001$).

The greatest density occurred in the summer at 20m in transect one which was 2,069 individuals m⁻² of which 936 m⁻² were the spirorbid worm *Protolaeospira stalagmia*. Members of the Mollusca contributed to the highest density followed by Annelida (fauna > 3mm) with 18,376 individuals and 8,956 individuals found within the 57 m² area sampled respectively (Figure 2.20).

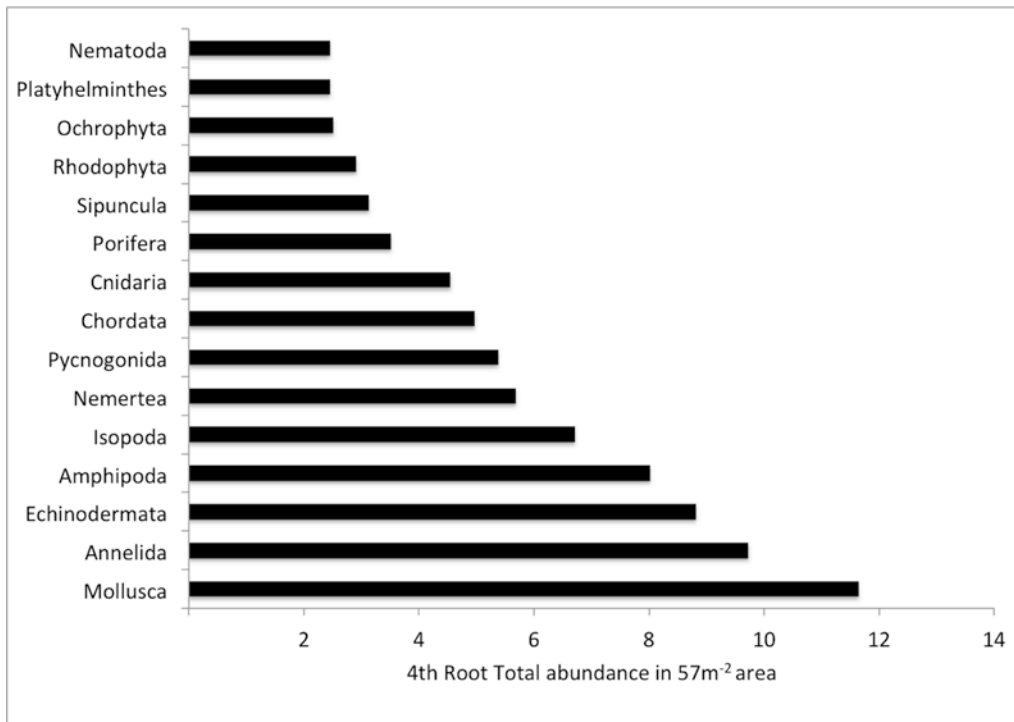


Figure 2.20. Total abundance for both austral summer and austral winter combined at Rothera Point in 2015. The data have been 4th root transformed and represent the 57m² sampled area.

Species diversity was not significantly different between seasons (GLM $F_{(1,17)} = 0.93$, $P = 0.354$ Figure 2.21) and there was also no interaction between season and depth (GLM $F_{(2,17)} = 0.34$, $P = 0.719$, Figure 21). However, species diversity was significantly different between depths (GLM $F_{(2,17)} = 37.66$, $P < 0.001$). Following a *post hoc* Tukey test, the diversity at each depth was significantly different ($P < 0.001$ Figure 2.21), 6m diversity was different to 12m ($P < 0.001$), 6m diversity was different to 20m ($P < 0.001$) and 12m diversity was different to 20m ($P < 0.001$).

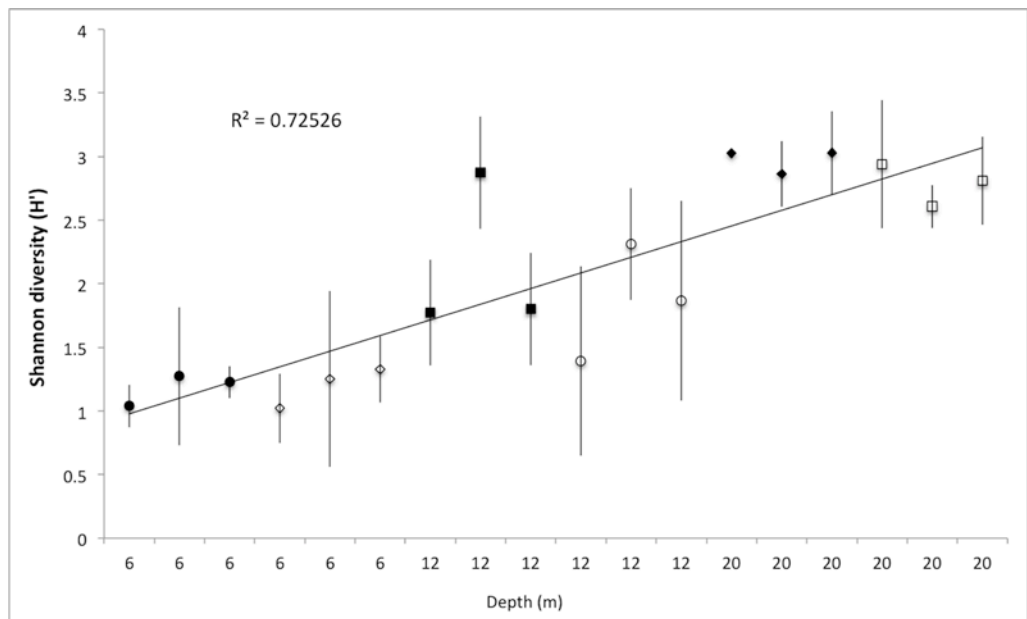


Figure 2.21. Species diversity with depth: 6m, 12m and 20m and season; austral summer (open data) and austral winter (filled data) at Rothera Point in 2015 error bars are +/- SD.

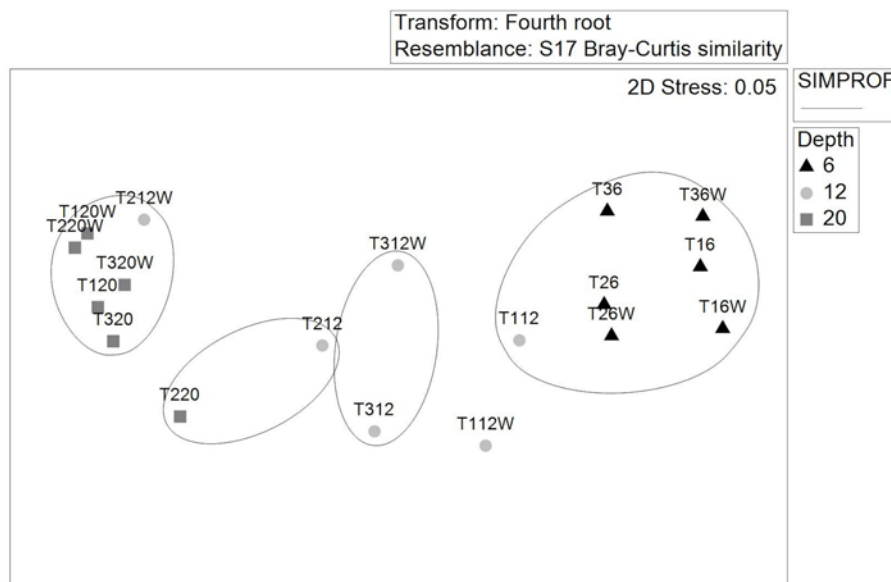


Figure 2.22. nMDS ordinations of density data (except Bryozoa) by depth and season. Bray – Curtis similarities (stress = 0.05) are calculated from 4th root averaged (for site) transformed data. The SIMPROF groups were based on 999 permutations with $P < 0.05$ significance and a cophenetic correlation of 0.846.

The nMDS ordination (Figure 2.22) of samples from the three depths and both seasons showed distinct clustering and some gradient separation by depth (ANOSIM $R = 0.786$). On contrast, there was no effect of season (ANOSIM $R = 0.074$) on benthic biodiversity. A similar result was found in the RELATE model in which the similarity matrix for summer was compared with that for winter ($\rho = 0.869$, $P = 0.002$ Table 2). A ρ value close to 1 indicates that the similarity matrices were similar, i.e. no difference between season. Vagile assemblages at 6m and 12m were dominated numerically by the limpet *Nacella concinna* and at 20m by the cushion star *Odontaster validus* (Table 2.3). The amphipod *Eurymera monticulosa* contributed most to the observed dissimilarity between depths of 6m and 12m, in which the mean abundance at 6m was 12 times higher than at 12m. The mollusc *Eatoniella caliginosa* contributed most to the observed dissimilarity between 6m and 20m, as none were found at 6m and there were on average 113 m⁻² at 20m. Finally, the presence of *Philobrya wandelensis* explained the most observed dissimilarity between 12m and 20m being five times more abundant at the deeper site (Figure 2.23).

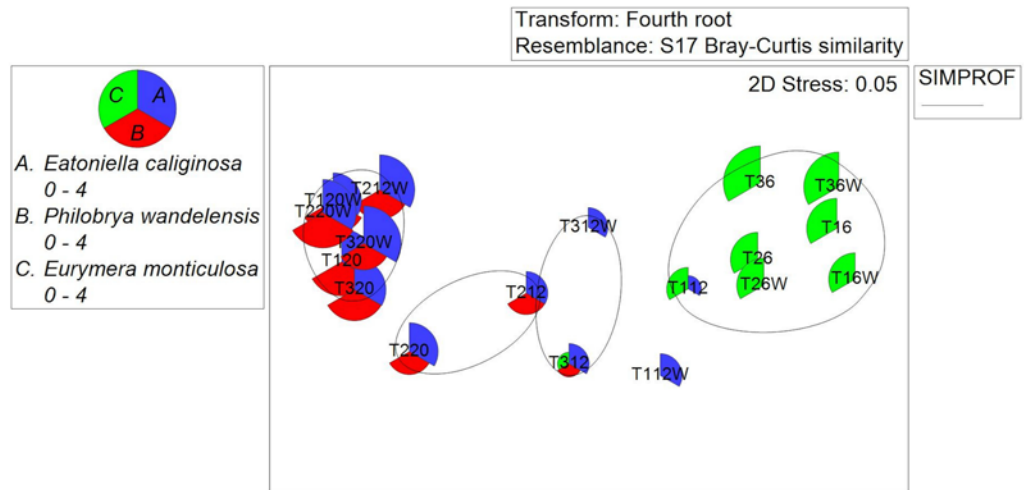


Figure 2.23. nMDS bubble plot of the three taxa that contributed most to the differences in community structure within the depth groups, using 4th root transformed average abundance data at 6m, 12m and 20m in both summer and winter.

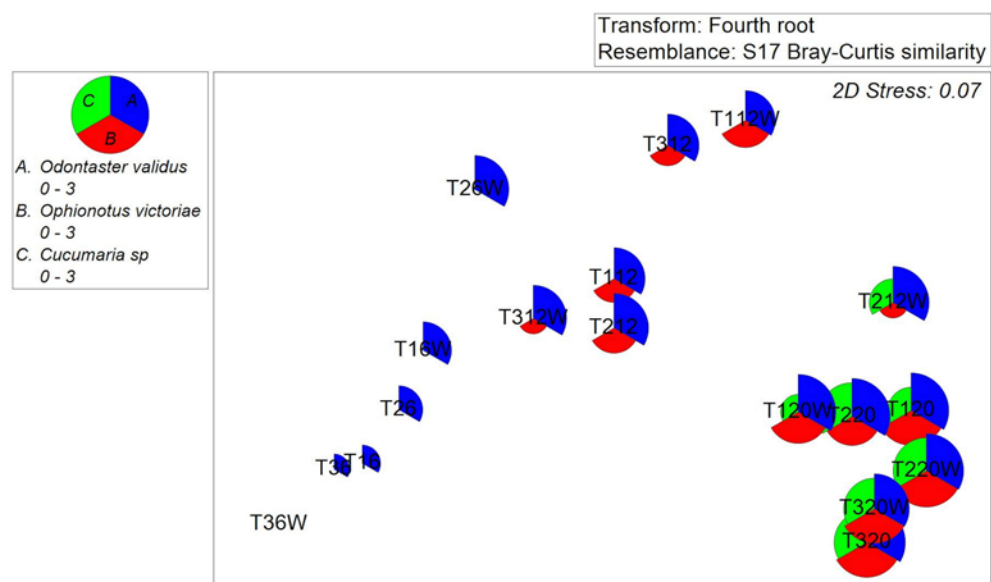


Figure 2.24. nMDS bubble plot of the Echinodermata taxa that contributed most to the differences in community structure within the depth groups, using 4th root transformed average echinoderm abundance data at 6m, 12m and 20m in both summer and winter.

Figure 2.24 shows an nMDS ordination of echinoderm only data at the three study depths across both seasons. There was no obvious separation between summer and winter which was confirmed by ANOSIM ($R = -0.099$) and RELATE models which showed a similar relationship between the summer and winter matrices ($\rho = 0.488$, $P = 0.006$ Table 2.3). However, there was a separation between depths ($R = 0.794$). The results from the SIMPER analysis based only on echinoderm data showed that the taxa most responsible for the observed differences between the different depths were *Odontaster validus*, *Ophionotus victoriae* and *Cucumaria* sp. (Figure 2.24 and Table 2.3). None of the different phyla within the samples showed a significant difference in community structure between summer and winter (ANOSIM $P > 0.05$). This was also confirmed using the RELATE model which showed no difference in the similarity matrix between summer and winter and that there is a similar relationship between the seasons. Furthermore there was no significant difference in primary consumers community structure between summer and winter (ANOSIM $R = -0.136$ Table 2.3).

Phylum	Transformation	Rank Similarities	SIMPROF (999 Permutations) P < 0.001	ANOSIM (2 WAY CROSSED) (999 Permutations)		SIMPER (Similarities) Season			SIMPER (Disimilarities) Depth			RELATE
				Season	Depth	6m	12m	20m	6m/12m	6m/20m	12m/20m	
All Phyla (except Bryozoans)	4th Root	B-C stress = 0.05	Pi 10.081	R = 0.074 P = 0.3	R = 0.786 P < 0.001	64.9 Similarity <i>Nacella concinna</i> 29.73	52.5 Similarity <i>Nacella concinna</i> 17.04	65.3 Similarity <i>Odontaster validus</i> 5.50	56.4 Dissimilarity <i>Eurymera monticulosa</i> 6.56	78.2 Dissimilarity <i>Eatoniella caliginosa</i> 3.68	54.3 Dissimilarity <i>Philobrya wandelensis</i> 3.14	$\rho = 0.869$ P = 0.002
Mollusca	4th Root	B-C stress = 0.04	Pi 9.871	R = -0.111 P = 0.8	R = 0.77 P < 0.001	87.9 Similarity <i>Nacella concinna</i> 82.88	58.0 Similarity <i>Nacella concinna</i> 50.94	71.2 Similarity <i>Eatoniella caliginosa</i> 15.77	44.2 Dissimilarity <i>Eatoniella caliginosa</i> 16.83	74.7 Dissimilarity <i>Eatoniella caliginosa</i> 13.77	50.6 Dissimilarity <i>Philobrya wandelensis</i> 11.62	$\rho = 0.886$ P < 0.001
Echinodermata	4th Root	B-C stress = 0.07	Pi 5.644	R = -0.099 P = 0.74	R = 0.794 P < 0.001	73.1 Similarity <i>Sterechinus neumayeri</i> 78.32	70.0 Similarity <i>Odontaster validus</i> 44.13	76.2 Similarity <i>Odontaster validus</i> 22.93	50.4 Dissimilarity <i>Odontaster validus</i> 26.07	68.8 Dissimilarity <i>Ophionotus victoriae</i> 17.59	45.1 Dissimilarity <i>Cucumaria sp</i> 15.89	$\rho = 0.488$ P = 0.006
Arthropoda	4th Root	B-C stress = 0.1	Pi 5.801	R = 0.16 P = 0.13	R = 0.798 P < 0.001	65.2 Similarity <i>Eurymera monticulosa</i> 37.51	47.3 Similarity <i>Prostebbingia sp</i> 27.93	61.7 Similarity <i>Cymodocella tubicauda</i> 27.75	58.8 Dissimilarity <i>Eurymera monticulosa</i> 21.26	81.5 Dissimilarity <i>Eurymera monticulosa</i> 14.06	59.1 Dissimilarity <i>Cymodocella tubicauda</i> 11.57	$\rho = 0.777$ P = 0.002
Porifera	4th Root	No Sig Pattern	n/a	No Sig Difference								
Ochrophyta	4th Root	No Sig Pattern	n/a	No Sig Difference								
Rhodophyta	4th Root	Some Pattern	Pi 5.801 P = 0.008	R = 0.025 P = 0.29	R = 0.204 P = 0.04	No species	No Species	17.2 Similarity <i>Kallymenia antarctica</i> 50.0	No Species	100 Dissimilarity <i>Rhodymenia subantarctica</i> 45.18	100 Dissimilarity <i>Rhodymenia subantarctica</i> 45.18	
Chordata	4th Root	B-C stress = 0.01 (with dummy variable)	No significant pattern	R = 0 P = 0.48	R = 0.521 P < 0.001	Cnemidocarpa verrucosa only identified specie within the phylla chordata						
Cnidaria	4th Root	No Sig Pattern	n/a	No Sig Difference								
Nemertea	4th Root	B-C stress = 0.05 (with dummy variable)	No significant pattern	No Sig Difference								
Annelida	4th Root	B-C stress = 0.09 (with dummy variable)	Pi 9.218	R = 0.173 P = 0.17	R = 0.449 P = 0.003	14.3 Similarity <i>Nereididae</i> UTU2 32.83	41.5 Similarity <i>Polynoidae</i> UTU1 29.47	61.9 Similarity <i>Thelepus cinnatus</i> 21.39	77.2 Dissimilarity <i>Polynoidae</i> UTU1 17.42	86.4 Dissimilarity <i>Protolaeospira stalagmia</i> 15.84	55.4 Dissimilarity <i>Protolaeospira stalagmia</i> 12.96	$\rho = 0.939$ P = 0.002
Bryozoa	4th Root	B-C stress = 0.03	Pi 11.182	R = -0.049 P = 0.53	R = 0.477 P = 0.007	12.1 Similarity <i>Fenestulina rugula</i> 77.21	29.4 Similarity <i>Fenestulina rugula</i> 63.45	66.1 Similarity <i>Fenestulina rugula</i> 26.72	81.5 Dissimilarity <i>Fenestulina rugula</i> 39.09	91.2 Dissimilarity <i>Fenestulina rugula</i> 20.00	67.8 Dissimilarity <i>Fenestulina rugula</i> 15.65	$\rho = 0.49$ P = 0.01
Primary consumers	4th Root	B-C stress = 0.05	Pi 11.607	R = -0.136 P = 0.82	R = 0.7 P < 0.001	67.5 Similarity <i>Nacella concinna</i> 57.1	44.2 Similarity <i>Nacella concinna</i> 30.48	67.3 Similarity <i>Fenestulina rugula</i> 15.78	56.7 Dissimilarity <i>Fenestulina rugula</i> 19.07	84.2 Dissimilarity <i>Fenestulina rugula</i> 12.15	62.5 Dissimilarity <i>Fenestulina rugula</i> 9.41	

Table 2.3. Results from Bray- Curtis similarity matrix with SIMPROF groups using 999 permutations at P < 0.05, results from a two way crossed ANOSIM with season and depth as factors and results from a SIMPER analysis on 4th root transformed average density data showing the species most contributing to the differences in depths.

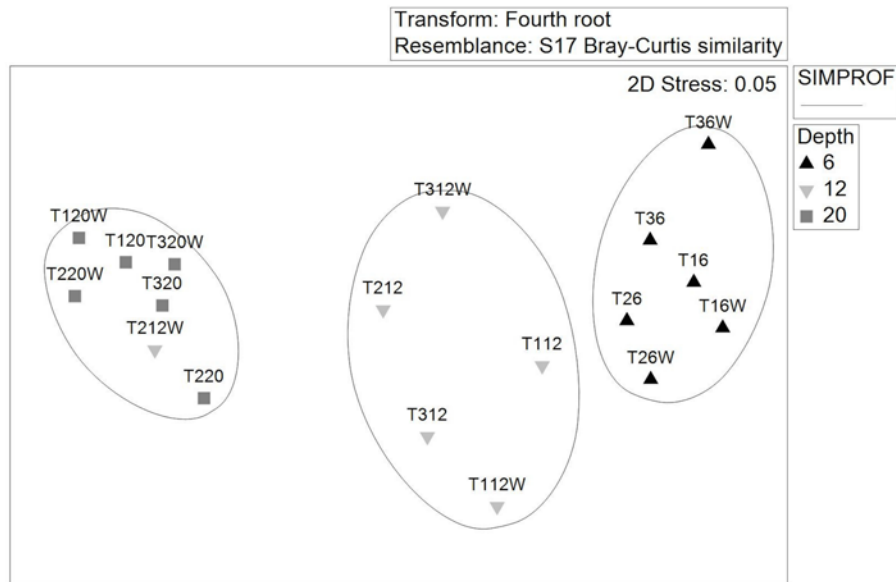


Figure 2.25. nMDS ordinations of biomass data (AFDM) by depth and season. Bray – Curtis similarities (stress = 0.05) are calculated from 4th root averaged (for site) transformed data. The SIMPROF groups were based on 999 permutations with $P < 0.05$ significance and a cophenetic correlation of 0.858.

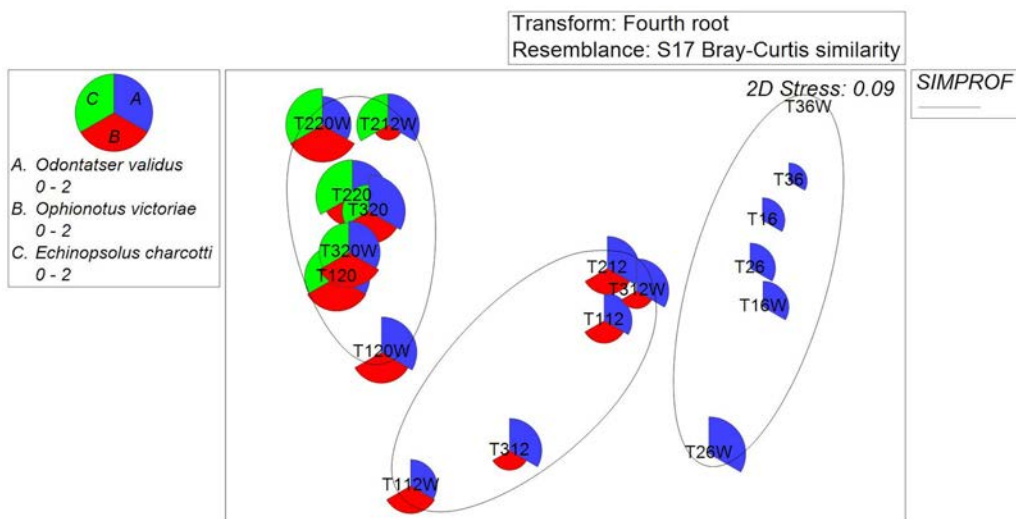


Figure 2.26. nMDS bubble plot using echinoderm only data for the three taxa with the most contribution to the differences in the depth groups, using 4th root transformed average biomass (AFDM) data at 6m, 12m and 20m in both summer and winter. Stress = 0.09.

The nMDS values for benthic biomass in summer and winter were generally well clustered i.e. visually there was no difference between seasons in biomass (Figure 2.25 AFDM g), which was confirmed by an ANOSIM with an R-value of 0.012 (Table 2.4). SIMPROF clusters groups based on 999 permutations and were significantly different $P < 0.001$. There was a difference in biomass (AFDM g) between the three study depths (ANOSIM $R = 0.815$ which is a good separation between groups). The organisms contributing most to the difference in biomass between depths (Table 2.4) were *Cryptasterias turqueti* and *Thelepus cincinnatus*. When only echinoderm data were compared (Figure 2.26) the three taxa responsible for most dissimilarity between depths were *Odontaster validus*, *Ophionotus victoriae* and *Echinopsolus charcoti*.

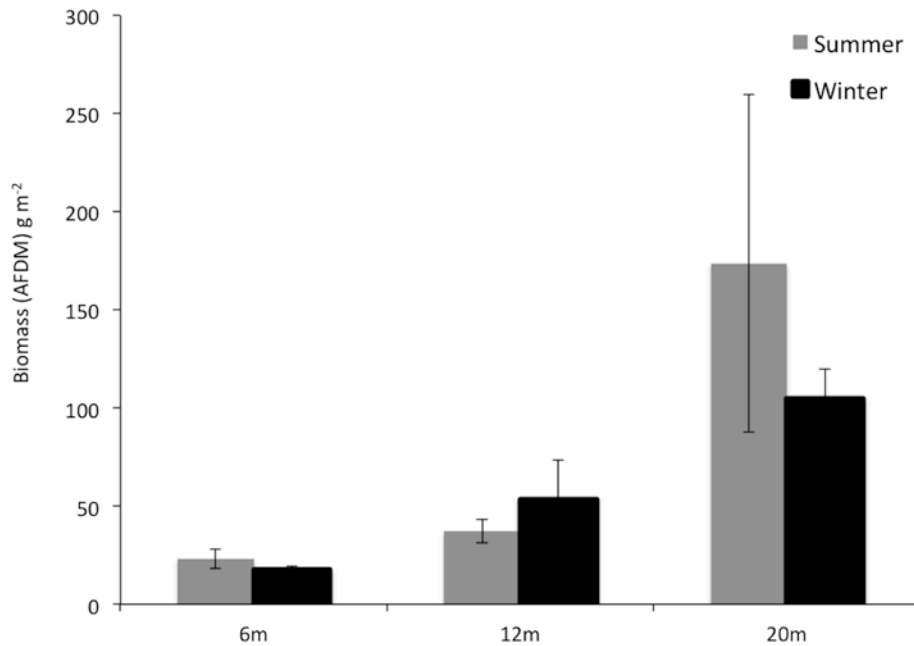


Figure 2.27. Mean biomass (AFDM) at three depths in the austral summer (grey bars) and the austral winter (black bars) means \pm SE.

There was a significant difference in biomass (AFDM g) between depths (GLM $F_{(2,56)} = 26.76$, $P < 0.001$ Figure 2.27) but no difference between seasons (GLM $F_{(1,56)} = 0.16$, $P = 0.693$) as shown in Figure 2.25. There were also no interactions between seasons and depths (GLM $F_{(2,56)} = 0.8$, $P = 0.921$). The species which contributed the greatest amount of organic carbon in a single sample was *Heterocucumis steineni* with 630.85 g m⁻² at 20m. However the mean biomass (AFDM) for *Heterocucumis steineni* at 20m was 36.33 g m⁻². The limpet *Nacella concinna* contributed the largest mean biomass (AFDM) at 6m and 12m, with 7.92 g m⁻² and 10.88 g m⁻² respectively. The annelid *Thelepus cincinnatus* contributed the highest amount of organic biomass (AFDM) at 20m of 21.73 g m⁻². Overall, the phylum echinodermata were the greatest contributors to organic carbon in the nearshore study site at Rothera Point, with mean values of 10.80 g m⁻² at 6m, 26.09 g m⁻² at 12m and 85.21 g m⁻² at 20m (Figure 2.28).

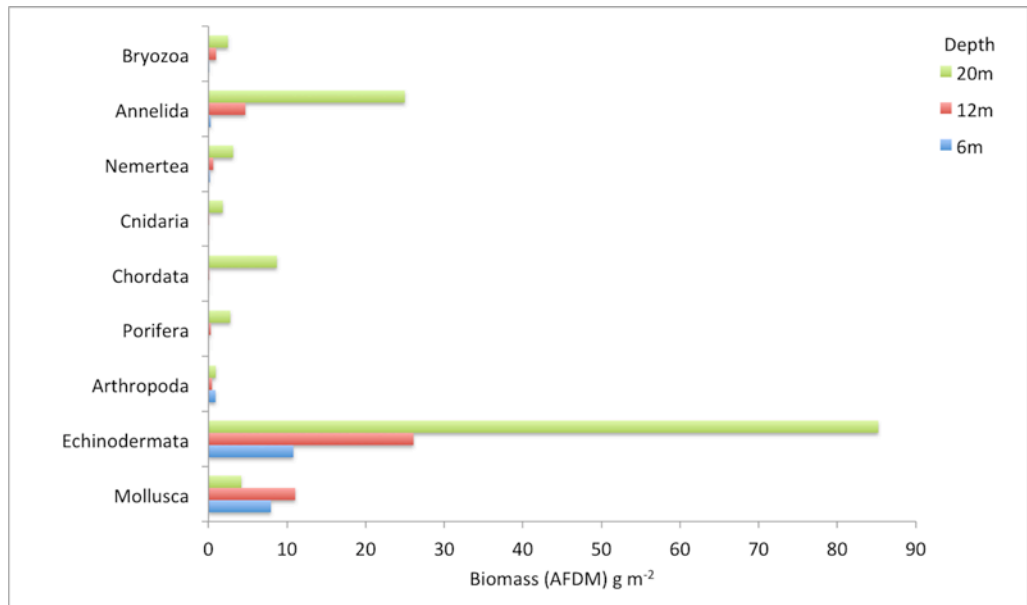


Figure 2.28. Mean biomass (AFDM) contributions of the main 9 phyla present at shallow sites on Rothera Point, at 6m, 12m and 20m

Phylum	Transformation	Rank Similarities (Based on 999 permutations)	SIMPROF (999 Permutations) P< 0.001	ANOSIM (2 WAY CROSSED) (999 Permutations)		SIMPER (Similarities) Season			SIMPER (Disimilarities) Depth			RELATE
				Season	Depth	6m	12m	20m	6m/12m	6m/20m	12m/20m	
All phyla	4th Root	B-C stress = 0.05 Graph	Pi 9.655	R = 0.012 P = 0.47	R = 0.815 P < 0.001	63.38 Similarity <i>Nacella concinna</i> 25.18	55.35 Similarity <i>Nacella concinna</i> 12.43	66.75 Similarity <i>Thelepus cincinnatus</i> 6.00	56.07 Dissimilarity <i>Cryptasteria s turqueti</i> 6.75	75.78 Dissimilarity <i>Thelepus cincinnatus</i> 4.45	50.61 Dissimilarity <i>Heterocucumis steineri</i> 4.84	p = 0.799 P < 0.001
Mollusca	4th Root	B-C stress = 0.04	Pi 8.122	R = 0.086 P = 0.26	R = 0.745 P < 0.001	92.20 Similarity <i>Nacella concinna</i> 88.56	64.57 Similarity <i>Nacella concinna</i> 67.36	71.20 Similarity <i>Nacella concinna</i> 21.09	32.17 Dissimilarity <i>Nacella concinna</i> 20.13	62.71 Dissimilarity <i>Nacella concinna</i> 13.19	46.95 Dissimilarity <i>Nacella concinna</i> 15.66	p = 0.824 P < 0.001
Echinodermata	4th Root	B-C stress = 0.09 Graph	Pi 5.228	R = -0.136 P = 0.89	R = 0.691 P < 0.001	69.14 Similarity <i>Sterechnus neumayeri</i> 73.63	62.90 Similarity <i>Odontaster validus</i> 40.69	74.69 Similarity <i>Odontaster validus</i> 18.77	50.19 Dissimilarity <i>Odontaster validus</i> 20.07	67.27 Dissimilarity <i>Ophionotus victoriae</i> 18.52	45.08 Dissimilarity <i>Echinopsolus charcoti</i> 16.90	p = 0.334 P = 0.03
Arthropoda	4th Root	B-C stress = 0.09	Pi 5.876	R = 0.099 P = 0.25	R = 0.844 P < 0.001	64.90 Similarity <i>Eurymera monticulosa</i> 39.39	47.43 Similarity <i>Prostebbingia brevi/ longicornis</i> 35.94	59.25 Similarity <i>Cymodocella tubicauda</i> 33.35	59.90 Dissimilarity <i>Eurymera monticulosa</i> 23.80	83.26 Dissimilarity <i>Eurymera monticulosa</i> 15.85	61.46 Dissimilarity <i>Prostebbingia brevi/ longicornis</i> 13.32	p = 0.763 P = 0.002
Porifera	4th Root	B-C stress = 0.05 (with dummy variable)	No significant pattern	No sig difference								
Chordata	4th Root	B-C stress = 0.01	No significant pattern	R = -0.136 P = 0.91	R = 0.492 P < 0.001	<i>Cnemidocarpa verrucosa</i> only identified species within the group Chordata						
Cnidaria	4th Root	B-C stress = 0.01 (with dummy variable)	No significant pattern	No sig difference								
Nemertea	4th Root	B-C stress = 0.04 (fix collapse)	No significant pattern	No sig difference								
Annelida	4th Root	B-C stress = 0.07 (with dummy variable)	Pi 8.47	R = 0.16 P = 0.13	R = 0.658 P < 0.001	10.74 Similarity <i>Thelepus cincinnatus</i> 30.00	43.46 Similarity <i>Polynoidea UTU1</i> 45.90	66.54 Similarity <i>Thelepus cincinnatus</i> 40.52	80.29 Dissimilarity <i>Polynoidea UTU1</i> 26.22	89.48 Dissimilarity <i>Thelepus cincinnatus</i> 32.34	56.40 Dissimilarity <i>Thelepus cincinnatus</i> 31.77	p = 0.794 P = 0.001
Bryozoa	4th Root	B-C stress = 0.04 (with dummy variable)	Pi 11.322	R = -0.148 P = 0.77	R = 0.576 P = 0.003	13.55 Similarity <i>Fenestrulina rugula</i> 84.32	30.60 Similarity <i>Fenestrulina rugula</i> 65.03	65.65 Similarity <i>Fenestrulina rugula</i> 28.92	81.82 Dissimilarity <i>Fenestrulina rugula</i> 43.82	92.17 Dissimilarity <i>Fenestrulina rugula</i> 21.92	66.24 Dissimilarity <i>Fenestrulina rugula</i> 17.35	p = 0.8 P = 0.003
Primary consumers	4th Root	B-C stress = 0.05	Pi 12.847	R = -0.074 P = 0.82	R = 0.704 P < 0.001	83.03 Similarity <i>Nacella concinna</i> 54.16	52.68 Similarity <i>Nacella concinna</i> 44.06	66.49 Similarity <i>Sterechnus neumayeri</i> 11.22	40.79 Dissimilarity <i>Sterechnus neumayeri</i> 16.68	73.13 Dissimilarity <i>Echinopsolus charcoti</i> 8.23	59.19 Dissimilarity <i>Echinopsolus charcoti</i> 8.34	p = 0.756 P = 0.002

Table 2.4. Results from Bray- Curtis similarity matrix with SIMPROF groups using 999 permutations at P < 0.05, results from a two way crossed ANOSIM with season and depth as factors and results from a SIMPER analysis on 4th root transformed average biomass data showing the species most contributing to the differences in depths.

2.4 Discussion

This is the first study to carry out seasonal benthic biodiversity and biomass measurements in the Antarctic. The total sample area of the current study (30m² in summer and 27m² in winter) may be considered a small area to represent the biodiversity of near shore shallow rocky communities (shallow sub littoral > 30m). However, few studies of nearshore biodiversity in the Antarctic have included as much replication or total area covered as that achieved here. Thus the current sample regime is the most thorough survey to date of an Antarctic near shore shallow water rocky community. Many similar morphotypes occur throughout the Antarctic shallows and, although the levels of brooding are high in the Southern Ocean, many of the most common marine species have planktonic larvae. As a result it has been suggested that the Antarctic fauna was predominantly circumpolar (Arntz, Gutt et al. 1997). However the recent biogeographic atlas of The Southern Ocean (De Broyer, Koubbi et al. 2014), which compiled the latest information across taxa from databases of georeferenced species reports, shows that there are strong local and regional differences. We are likely to get a better idea of true spatial variability in assemblages as molecular genetics becomes more widely and rigorously applied in polar biodiversity studies.

The samples in the current study comprised 42,861 individuals, which represented 148 species within the 57m² area (Figure 2.20). These figures are similar to other Southern Ocean regional locations, e.g. Barnes, Linse et al. (2008) found 163 species in their survey of a similar area at Deception Island, Antarctica, and Barnes, Kaiser et al. (2009) reported 158 species from 81 m² surveyed in the South Orkney Islands. Across a broader scale, Gutt, Sirenko et al. (2004) estimates there are the between 11,000 and 17,000 species over the entire Antarctic shelf. Uncovering of cryptic speciation, however, are likely to increase the number significantly (Sands, O'Hara et al. 2015). Life is typically abundant in the Southern Ocean, but measuring diversity is difficult because of high population and community patchiness and a complex hierarchy of scales of spatial variation (Gray 2001, Teixido, Garrabou et al. 2002, Thrush, Hewitt et al. 2010).

The patchy distribution, a minimum of 304 individuals m^{-2} and maximum of 2,069 individuals m^{-2} in samples from 20m of Antarctic marine benthos was evident in the current study. It is therefore important to have a high number of replicate samples to allow for this patchiness and to better estimate local density, species richness, diversity and biomass of a sample area. Species accumulation curves for fauna at our study site neared asymptotes (Figure 2.17), which suggests that the majority of local benthic macro invertebrates were sampled (within that habitat). Some megafauna such as the starfish *Labidiaster radiosus* (which can be over 40cm across) are unlikely to be captured by 0.25 m^2 quadrat sampling, and for such species a better sample unit would be 1 m^2 quadrat. There are several other large taxa that are unlikely to be sampled using methods here because they are rare and cover large areas. This study also did not include fish because they are more mobile. However this method with 0.25 m^2 quadrat and suction sampler aided sampling of many other benthic marine invertebrates, which are not large enough for visual and hand collection sampling. This is important as key taxa sampled this way; the amphipod *Eurymera monticolosa*, the gastropod *Eatoniella caliginosa* and the bivalve *Philobrya wandelensis* (collected using the suction sampler) were responsible for most of the dissimilarity in community structure between depths (Table 2.3).

SCUBA diving techniques mean sampling can be more targeted and less destructive than grabs and trawls, with little damage to organisms allowing for higher quality taxonomic identification within the 0.25 m^2 study areas. However, SCUBA diving is a spatially limited technique, partly due to safety restrictions on time and depth under the water.

With a 25 minute dive time at 20m, a 0.25 m^2 quadrat was only just achievable and allowed for greater replication. Complete removal of organisms was necessary to carry out biomass (AFDM) measurements, which for a sedentary organism such as *Thelepus cincinnatus* was easier by hand and therefore required SCUBA. *Thelepus cincinnatus* was responsible for most of the dissimilarity in biomass (AFDM) between depths (Table 2.4).

Latitude	Location	Year	Season	Depth(m)	Substrata	Mean number of taxa	Mean Density m ⁻²	Mean Biomass g m ⁻²	Mean Diversity (H')	Reference
Polar	King George island, Admiralty Bay	2001	Summer	12	Soft	8	119.5	177.68(WM)	1.61	Echeverria, Paiva et al 2005
Polar	King George island, Admiralty Bay	2001	Summer	25	Soft	6	171.5	280.58 (WM)	0.94	Echeverria, Paiva et al 2005
Polar	King George island, Admiralty Bay	1999	Winter	12	Soft	7	60.66	134.67 (WM)	1.83	Echeverria, Paiva et al 2005
Polar	King George island, Admiralty Bay	1999	Winter	25	Soft	7	145.78	171.61(WM)	1	Echeverria, Paiva et al 2005
Polar	King George Island, Potter Cove	1998	Summer	2000	Soft		730	6 (AFDM)		Piepenberg, Schmid et al 2002
Polar	King George Island, Potter Cove	1998	Summer	100-200	Soft		>14 000	90 (AFDM)		Piepenberg, Schmid et al 2002
Polar	NW Spitsbergen, Arctic	1991-2012		2000-5500	Soft		552	0.06(WM)		Degen, Renet et al 2015
Polar	Fram Strait, Arctic	1991-2012		2500-4200	Soft		326	0.05(WM)		Degen, Renet et al 2015
Polar	Amundsen Basin	1991-2012		3700-4500	Soft		61	0.04(WM)		Degen, Renet et al 2015
Tropical	Port phillip Bay, Australia			2 - 23	Soft	14	269.4			Gray et al 1997
Tropical	Western Port, Australia			0 - 10	Soft	47	35			Coleman et al 1978
Tropical	Kingston Harbour, Jamaica			6 - 16	Soft	24	10	24.6(WM)	4.3	Wade 1972
Tropical	Hong Kong	76/77		13-23	Soft	37	101.4	35.2(WM)	3.67	Shin, Thompson 1982
Tropical	New Caledonia				Soft			13(AFDM)		Chardy and Clavier
Tropical	Farasan Island, Red Sea	2014		5-138	Soft	27	140		2.7	Ellis, Anlauf et al 2017
Polar	Adelaide Island, Rothera	2015	Summer	6	Hard	12	478	23.24(AFDM)	1.2	Current study
Polar	Adelaide Island, Rothera	2015	Summer	12	Hard	20	558	37.23 (AFDM)	1.9	Current study
Polar	Adelaide Island, Rothera	2015	Summer	20	Hard	43	1111	173.68(AFDM)	2.79	Current study
Polar	Adelaide Island, Rothera	2015	Winter	6	Hard	10	507	17.44(AFDM)	1.18	Current study
Polar	Adelaide Island, Rothera	2015	Winter	12	Hard	26	679	53.47(AFDM)	2.15	Current study
Polar	Adelaide Island, Rothera	2015	Winter	20	Hard	49	1186	104.73(AFDM)	2.97	Current study
Polar	Terra Nova Bay, Ross Sea	89/90	Summer	6	Hard		13 498	167.02 (WM)	2.2	Gambi, Lorenti et al 2004
Polar	Terra Nova Bay, Ross Sea	89/90	Summer	12	Hard		19 206	479.70(WM)	2	Gambi, Lorenti et al 2004
Polar	Terra Nova Bay, Ross Sea	89/90	Summer	16	Hard		9026	169.70(WM)	1.4	Gambi, Lorenti et al 2004
Tropical	North Aegean Sea	97/98	Summer	15	Hard	79	1412		4.1	Antoniadou, Chintiroglou et al 2004
Tropical	North Aegean Sea	97/98	Summer	30	Hard	88	1440		3.98	Antoniadou, Chintiroglou et al 2004

Table 2.5 Mean density (individuals m⁻²), mean taxa (species m⁻²), mean biomass (g m⁻²) and mean diversity (H') of benthic communities in various Antarctic, Sub Antarctic and Tropical areas.

Historically, rocky sub-tidal communities are among the least studied of all marine biota, especially with respect to their ecology (Fraschetti, Bianchi et al. 2001). Macro and mega benthic diversity (H') is higher on hard substrata compared with soft sediments in Antarctica, which could be due to a higher species richness on hard substrata or because many species live in sediments (meiofauna) rather than on them (Table 2.5). Rocky sub-tidal communities generally include a wide range of species with very different life cycles and recruitment strategies from those displayed by soft-bottom assemblages from the same regions at similar depths (Albertelli et al. 1999). In contrast, meiofauna can have a 7 fold higher density in soft sediments compared with hard substrata (Danovaro and Fraschetti 2002). Vausse, Morley et al. (in press) conducted a study of biodiversity in soft sediments from two sites at Rothera Research Station and although the density within the soft sediments was greater, it was only 2 fold greater than the density found on hard substrata.

Hard substrata have much lower interstitial space, so meiofauna have a reduced potential for colonisation. Polychaetes seem to dominate soft sediment samples (Danovaro and Fraschetti 2002) in terms of species richness compared to hard substrata which are dominated by molluscs (Figure 2.20). Ashton, G (2017 in press), Bowden, Clarke et al. (2006) and Stanwell-Smith and Barnes (1997) showed that bryozoans and spirorbid polychaetes were the dominant colonizing taxa both in terms of numbers and areas covered on hard substrata. However, those studies investigated pioneer species and newly colonized areas. This study evaluated biodiversity of flora and fauna larger than 3mm in size and would not have included the smaller spirorbid worms, which were plentiful.

Antarctic near shore hard bottom diversity (H') from this study was almost half of the diversity (H') found on tropical near shore hard bottoms. Mean density from this study was similar to tropical hard substrata, however the mean number of taxa was significantly higher in the tropics (Table 2.5). Biodiversity is distributed heterogeneously across the Earth. It has long been acknowledged the species richness of most groups of organisms increases from higher to lower latitudes (Gaston 2000). There are a large number of possible mechanisms that could underpin the patterns reported above:

Disturbance: the nearshore shallows are very regularly and catastrophically disturbed by ice scour. Areas of seabed from 5-25m deep which were monitored at Rothera showed that 30% was hit every year and only 7% was not hit in a decade (Barnes 2017).

Size of area: The Pacific Ocean alone is 8x the area of the Southern Ocean (Eakins and Sharman 2010). Gaston (2000) showed that the number of species increases with area. The nearshore ice shelf free shallow coastline in Antarctica covers 5,468 km, 2.7% of global coastlines (Smale, Barnes et al. 2008), which is far less coastal length than is available in the tropics. The total area of the Southern Ocean is about 34.8 million km². Of this, up to 21million km² is covered by ice at the winter maximum but only about 7 million km² is covered at the summer minimum (Gloersen, Campbell et al. 1992).

Age of area: the tropics, especially in very shallow benthic areas, are much older than the Southern Ocean. This is because the continental shelf was covered by an ice sheet at the last glacial maximum 15 million years ago (Clark, Dyke et al. 2009) and disturbance from ice scour in the Southern Ocean (Chapter 3) maintains ecosystems in early successional stages. For example, 12 years after the disintegration of the Larsen A ice shelves, the low faunal density, low species richness, and occurrence of deep-sea taxa testified to the former oligotrophic conditions, but the presence of pioneer species such as fast-growing ascidians *Molgula pedunculata* suggested an early successional stage of the colonization process (Gutt, Barratt et al. 2011).

Exploration: greater sampling and taxonomic effort in the tropics compared with the polar regions.

Niches: Tropical communities contain disproportionately more specialized and rare species than assemblages at higher latitudes (Brown 2014), increasing the mean number of taxa present. This could be due to tropical habitats being better studied and the number of rare species being proportional to sampling effort.

Across taxa, Antarctic waters are generally as rich as other non-coral reef areas globally (Clarke and Johnston 2003). Within taxa, richness of Antarctic waters is highly variable from super rich in some (e.g. Pycnogonida, see Munilla and Soler Membrives 2008) to poorly represented (decapods, bivalves and teleost fishes, Clarke and Johnston (2003)). The first comprehensive biodiversity survey of the South Orkney Islands, near the tip of the Antarctic Peninsula, revealed that they are home to more species of marine animals than the Galapagos (Barnes, Kaiser et al. 2009).

Species richness, faunal abundance, diversity and biomass (AFDM) all significantly differed across depths (GLM $P < 0.05$). This was an expected result from visual observations and previous Antarctic benthic studies. For example, Barnes and Brockington (2003) found faunal abundance increased logarithmically from <100 to >10000 individuals m^{-2} from the intertidal to 35 m respectively. Significant differences between depths were also found in the soft sediment survey at King George Island, Antarctica by Echeverria, Paiva et al. (2005). Furthermore, zonation patterns and differences between depths were reported in a benthic survey at Terra Nova Bay, Ross Sea, Antarctica (Table 5) (Gambi, Lorenti et al. 1994). Antarctic studies often find distribution at the community and species levels reveals a well-defined zonation pattern as a function of depth (Gambi, Lorenti et al. 2004, Barnes, Linse et al. 2008). A similarly strong intensity of faunal depth horizons was previously reported at McMurdo Sound, a high latitude Antarctic locality (Dayton, Robilliard et al. 1970).

Biomass, in many studies along the Antarctic and sub Antarctic coasts, has been generally reported only as wet mass because of the taxonomic importance of the material collected (Muhlehardt- Siegel 1988). However this makes it difficult for direct comparisons between studies due to differing methods of obtaining wet mass. This study measured AFDM as wet mass does not provide an accurate measurement of organic carbon (Table 2.5). Biomass measurements were consistent with those of faunal density in that biomass increased with depth (Figure 2.28). There was high variability (large standard deviation) among replicates (for example at 20m on the same transect samples T3203 and T3201 had 49.7 g m^{-2} and 937.5 g m^{-2} respectively), which was due to patchiness within the environment i.e. time since last ice scour and patchiness within certain species.

Wet Mass (WM) is not an accurate measure of organic biomass as some benthic invertebrates such as the holothurian *H. steineni* contain ~80% of water (Nicol 1967). This was substantiated by data from this study using WM and DM measurements for *H. steineni*, On average this species was 86% +/- SE 0.006 water. DM is also not an accurate measure of organic biomass, for example holothurians also have a high inorganic content due to the presence of calcium carbonate ossicles in the body wall. Cushion stars such as *O. validus* have a high amount of inorganic carbon within their skeletons. The dry mass of the urchin *S. neumayeri* is predominantly skeletal carbonate with high water content as well (~ 80%) (Brockington, Clarke et al. 2001). AFDM measures the purely organic material present in the specimen and thus provides a better measure of metabolically active tissue than wet mass or dry mass (Fraser, Peck et al. 2004).

Barnes and Brockington (2003) found the echinoid *S. neumayeri* was the principal cause of high biomass values, as it dominated biomass at all sub-tidal depths studied. However their measurements were of WM and not AFDM. The current study showed that on average, individual *H. steineni* contributes 30% more organic carbon than *S. neumayeri* (mean AFDM of 13.84 g m^{-2} and 5.61 g m^{-2} respectively). With the exception of the polychaete *Thelepus cincinnatus*, the gastropod *Nacella concinna* and the ascidian *Cnemidocarpa verrucosa*, the

majority of biomass at Rothera came from echinoderms (Figure 2.29). This seems to be the case elsewhere in nearshore habitats and also in deeper water and is generally characterized mainly by high contributions of echinoderms (Brey and Gerdes 1997).

Seasonality, productivity and ice scour are three main factors affecting nearshore shallow water benthic biodiversity. Species richness, density, diversity and biomass on the near shore shallow water rocky habitats at Rothera Point were not significantly different between seasons. This study was restricted to only one year. Sampling in subsequent years would enable more robust testing of seasonal differences, but is logistically difficult.

Other comparisons of polar benthic communities over time have been carried out, most along the Western Antarctic Peninsula. Studies of benthic megafauna from the soft bottoms of the shallow coastal zone of Martel Inlet (Admiralty Bay, King George Island, South Shetland Islands) also found little variation in community composition with time. That work examined three non-consecutive sampled years in the same area, although only in summer (Nonato, Brito et al. 2000). Studies of benthic meiofauna and macrofauna from the soft seabed sites around Rothera Point also found no difference in community composition with different seasons (Vausse, Morley et al. 2018 in press).

The lack of temporally significant variation in benthic megafauna at King George island, Antarctica in 12 months suggests that megafauna are not strongly affected by the winter decrease in primary production (Echeverria, Paiva et al. 2005) organisms are not very mobile. Lower winter metabolic rates mean lack of food or poor quality food can sustain the organisms (Chapter 4).

Suspension feeding and a sessile nature are positive attributes in terms of maintaining of low metabolic costs in a cold water, food limited environment and may aid establishment (Peck, Brockington et al. 1997). Studies of ecological processes require considerable time, and in Antarctica, where such processes take place slowly, these studies are necessary long-term (Clarke 1996). No

consistent differences in meiofaunal and macrofaunal density, diversity or composition among the four seasons were detected in an Arctic fjord either (Wlodarska-Kowalczyk, Gorska et al. 2016). However, significant temporal changes of meiofaunal density were observed on both hard and soft substrata, with higher densities in spring to summer and lowest abundance in winter (Wlodarska-Kowalczyk, Gorska et al. 2016). Other studies of benthic biodiversity have been carried out in the Antarctic (Table 2.5) although due to logistical constraints these are mainly summer only. Interannual or seasonal changes in the density or biomass of the megafauna community at King George Island were not significant either, although as with this study, there were significant differences between depths (Echeverria, Paiva et al. 2005).

Ice disturbance at shallow depths in Antarctic coastal zones exerts a strong selective pressure on organisms (Chapter 3), and the effects on benthic community structure have been well documented (Peck, Brockington et al. 1999, Smale 2007, Smale, Barnes et al. 2007, 2008, Smale, Brown et al. 2008). Differences in faunal density and biomass among depths and variability among replicates are shown in the cluster diagrams (Figure 2.22 and 2.26). The samples at 12m (T212W) clustered with those at 20m largely because the T212 sample site had a high species diversity (Figure 2.21).

The organic carbon content of Antarctic sediments tends to be rather low even though sedimentation pulses from surface phytoplankton blooms can be important in summer (Dayton 1990). This suggests that the benthic community may be an important carbon sink (Dayton 1990, Barnes 2015). Elemental composition of tissues of six species of Antarctic marine invertebrates was 49 to 60% carbon (Clarke 2008).

In this study, benthic sessile primary consumers were rich and abundant (total number = 25,952 of the 42,861 individuals and total biomass = 2,106.47g of the total 3,936.27g in 57m² area). Amongst this functional group, *Fenestrulina rugula*, a cheilostome bryozoan, was an important contributor to the dissimilarity in primary consumers between depths (Table 2.3). Previous work

at the same locality showed that their mortality rates were very high; increases in ice scour in the Antarctic shallows mean that few *F. rugula* survived more than 2 years (Barnes and Souster 2011).

F. rugula and other encrusting organisms are important for carbon accumulation (and probably sequestration); however ice scour can grind up benthos. Such ice scour recycles carbon by releasing carbonate back into the water column, or in the case of soft-bodied benthic sessile or sedentary primary consumers such as the holothurian, *H. steineni*, recycling of organic carbon. Ice scour in near shore Antarctic shallows can have a big impact on carbon accumulation and immobilization levels in the shallow zoobenthos, but becomes less important by 100m depth (Barnes 2017).

The biomass of *Echinopsolus charcoti* (holothurian) contributed to the dissimilarity in primary consumer biomass between depths (Table 2.4). These species may prove to be important in terms of carbon accumulation (Barnes 2017) but being soft bodied it remains unclear how much of that is genuinely sequestered.

Total biomass (AFDM) for the 57m² area of nearshore benthic hard substratum in this study was 3,936.3g, of which primary consumers contributed 2106.5g. Biomass is related to food availability and for Antarctic benthic marine invertebrates food availability can be seasonal. Food supply is already being affected by climate change, for example the magnitude of the spring phytoplankton bloom at Rothera is much reduced following winters with the lowest sea ice cover (Venables, Clarke et al. 2013). However, reduced sea ice cover also increases the duration of the phytoplankton bloom, so even though the magnitude of the bloom may be lower, longer duration means a longer period of feeding and therefore potentially more growth and accumulation of biomass. The Southern Ocean has a high level of productivity close to the coast, which is dense but brief (8 – 10 weeks), limiting the amount of time for organisms to convert energy into biomass (Chapter 4). In contrast the tropics typically have a roughly constant production throughout the year (Brown 2014,

Clarke 1988). However, peak productivity is lower in the tropics due the presence of a permanent thermocline which prevents mixing between surface waters and nutrient rich deep waters compared with in the Southern Ocean.

2.5 Conclusion

Overall, the distribution of Antarctic benthos appears to be governed by ice impact, food supply and substratum. Benthic biodiversity can also be correlated with depth as an indirect effect as light levels are reduced which reduces primary production. Ice scour, however is a far bigger factor explaining patterns of benthic biodiversity with depth as there is a massive change in both biodiversity and biomass between 5m and 20m. Although there was no significant difference in biomass (AFDM) and biodiversity between seasons in near shore rocky substrata at Rothera, this is the first study measuring AFDM of benthic communities on hard substrata in both summer and winter in the Antarctic. Benthic marine Antarctic invertebrates grow slowly and generally take longer than lower latitude species to reach reproductive maturity, therefore there is a need for long-term benthic biodiversity monitoring to be able to assess future change. One of the earliest taxa used to investigate trends in marine biodiversity were the Mollusca, which were the most abundant taxa within the sub-Antarctic and Antarctic Peninsula (Linse, Griffiths et al. 2006).

Although hard substrata are in near shore coastal waters less common than soft sediment, their associated fauna are more diverse and have a higher species richness. Under future climate change scenarios, glaciers will continue to retreat, more near shore shallow habitat will be available for colonisation and there will possibly be an increase in species richness with area. However, there will also be less winter fast ice and a higher frequency of ice shelf collapse, which will increase ice scour of near shore benthic communities. This study quantified biodiversity and biomass of near shore rocky habitats in Antarctica to provide a baseline for monitoring future change. This study also measured biodiversity across all phyla providing a very valuable baseline data set rather than for one specific animal group, so we can now get a better perspective of the biodiversity in the near shore Sublittoral areas around Rothera Point. Future

research necessary would be to be able to identify Antarctic polychaetes to at least genus level rather than using molecular methods which meant the polychaete IDs were at family level. More seasonal studies on hard rock benthic communities in Antarctica needed at frequent intervals to assess the effect of seasonality long term, if any.

Chapter 3 – Benthic assemblage
biodiversity and biomass, changes
with depth and time on hard
substratum at Adelaide Island,
Antarctica

Chapter 3 Benthic assemblage biodiversity and biomass, changes with depth and time on hard substrata at Adelaide Island, Antarctica

3.1 Introduction

In Chapter 2, I reported a quantitative assessment of the biodiversity and biomass of near shore rocky habitats in Antarctica during both the austral summer and winter. This was primarily to provide a baseline for monitoring of future change under predicted IPCC (Intergovernmental Panel on Climate Change) scenarios. In this chapter I investigate the importance of monitoring future change by comparing biodiversity and biomass over time with a survey carried out at the same location in the austral summer of 1998 (Barnes and Brockington 2003). Therefore the method is comparable with that used in the 1998 survey, and a sub set of the method used in Chapter 2. I investigate benthic biodiversity and biomass (wet mass WM not ash free dry mass AFDM as used in Chapter 2) changes over time with depth using the same sampling location of Cheshire Island (Figure 3.1). Chapter 2 provided a detailed biodiversity survey including the use of a suction sampler, also incorporating depth as a factor but examining changes over season whereas this chapter focuses on change over time.

The shallow waters around the Western Antarctic Peninsula (WAP) have been one of the fastest warming places on earth, with a shallow water temperature increase of 1°C in the last half of the 20th century (Meredith and King 2005). The IPCC confirmed that mean global warming was 0.6 +/- 0.2 °C during the 20th century with the WAP warming more rapidly than the rest of Antarctica (Vaughan, Marshall et al. 2003). The WAP region has among the highest recorded increases in air temperature, glacial retreat, ice shelf and sea ice loss (Turner, Bindshadler et al. 2009). Rothera is situated on Adelaide Island (central WAP) and is well placed to study how shallow coastal species are responding to climate change as it is situated on the Antarctic peninsula an area which has warmed faster than

anywhere else on earth. Climate change is likely to influence biodiversity through many processes, such as direct temperature effects on organisms (Peck, Webb et al. 2004, Peck, Clark et al. 2009), oceanographic shifts (Young, Goldman et al. 2015), ice shelf collapse (Fillinger, Janussen et al. 2013), glacial retreat (Sahade, Lager et al. 2015), changes in the dynamics of sea ice (Atkinson, Siegel et al. 2004, Clark, Marzinelli et al. 2015) and ice scour (Smale and Barnes 2008, Smale, Barnes et al. 2008, Smale, Brown et al. 2008, Barnes and Souster 2011, Barnes 2017a). In Antarctica alone, there are ~5,500km of ice-shelf-free shallow coastline (Smale, Barnes et al. 2008), most of which could be impacted by one of the ~300,000 icebergs (Orheim 1987) that float around the Southern Ocean. The Antarctic ice shelves produced 70,000 icebergs (greater than 10 m wide) between 1981 and 1985 (Lien, Solheim et al. 1989).

One of the longest directly observed Antarctic records of annual fast ice duration from the 1980s to the present day is available for the Rothera area (Barnes and Souster 2011). This has shown a reduction in winter fast ice over this time. Intensity of ice scour is negatively correlated with the duration of the winter fast ice season (Smale, Barnes et al. 2008). Increased scouring of the sea bed has led to higher mortality of benthic organisms, with implications for the regions biodiversity (Peck, Brockington et al. 1999, Barnes and Souster 2011). Moreover Smale et al. (2007) reported massive reduction in species richness due to disturbance from ice scour impact. However the effects differed with organism size, species and substratum (Peck, Brockington et al. 1999). Relative abundance of low mobility groups was greater at low disturbance levels and abundance of secondary consumers was greater at high disturbance levels (Smale et al. 2008).

Disturbance is a key structuring force influencing shallow water communities at all latitudes (Smale, Brown et al. 2008). Open water conditions in polar summers incorporate some of the highest disturbance frequencies from ice berg scour in the natural world. Gutt and Starmans (2001) suggested that iceberg scouring to be one of the five most

significant impacts on any ecosystem on Earth. Although there are natural disturbance events that rival ice-scouring in either frequency or catastrophic power at lower latitudes in coastal systems, none do high frequency and high power nor across such a wide depth range as found on the WAP (Brown, Fraser et al. 2004). Only intensively trawled seabeds' have come close to the frequency and destructive power of ice scour from grounding icebergs (Barnes and Conlan 2007). Ongoing warming is likely to sustain fast ice losses and thus iceberg disturbance is likely to increase in near shore polar waters. Some of the world's most intensely disturbed faunas may soon suffer even more disturbance (Barnes 2017a).

In a Web-of-Science search (August 2017), over 6,500 publications included the words *diversity* and *disturbance*. The term 'disturbance' is used in Ecology to refer to a wide variety of phenomena e.g. fires, storms, diseases, volcanic eruptions, earthquakes, contaminant spills, land clearing, ice scour and dredging, among many others (Dornelas, Soykan et al. 2010). Therefore, it is not surprising that definitions of disturbance are wide and inclusive. Arguably the simplest is, "a discrete, punctuated killing, displacement, or damaging of one or more individuals (or colonies) that directly or indirectly creates an opportunity for new individuals (or colonies) to become established" (Sousa 1984). Physical disturbances by their nature occur by chance with different frequencies and intensities, due to varying spatial and temporal factors, making them, and the associated responses, very hard to predict (Sugden 2007). It is now well established that disturbance is a major source of temporal and spatial heterogeneity in the structure and dynamics of natural communities, and an agent of natural selection in the evolution of life histories (Pickett and White 1985).

Baseline studies need to be able to quantitatively measure biodiversity in order to address such fundamental questions as how biodiversity changes through time (Gaston 2000). Thus the value of the study recorded in this chapter is enhanced by a previous biodiversity assessment at the same site in 1998 (Barnes and Brockington 2003). Understanding how biodiversity

has arisen and how it has changed in the past, provides clues to interpreting its present and future structure (Gaston 2000). Scientific research into the Antarctic marine ecosystem only began in the mid-nineteenth century. Expeditions such as those carried out by HMS Challenger, RV Belgica, and RRS Discovery were among the first to collect and catalogue the benthos (Griffiths 2010), and scientific knowledge of marine communities has been advanced by long term studies of polar marine biodiversity (Griffiths 2010).

Of all the species that live in the sea, only about 2% of marine species permanently live in the water column, the remainder living on or in the sea bed (Gaston and Spicer 2003). There are concerns over the future of biodiversity in some habitats and ecosystems, and there is a need to determine its current status and predict its likely response to environmental change. Biodiversity research has increased around the Antarctic and Southern Ocean in recent decades. In part the increasing interest has been driven by the realisation that any fundamental quest to understand life's diversity requires exploration of the polar regions (Convey, Chown et al. 2014). Therefore while the need for benthic biodiversity assessments in Antarctica is clear to the polar ecologist it should also be to scientist and politicians more generally.

Antarctica has been suggested as a powerful natural laboratory for studying biodiversity, evolution and the impacts of climate change. The Antarctic marine biota is rich and distinctive compared with many areas elsewhere in the world (www.SCAR-marBIN.be). Many groups of Antarctic benthos exhibit a high degree of endemism (for marine taxa this ranges from 35 to 90% of species; Arntz, Gutt et al. 1997, Griffiths 2010). Shallow water coastal ecosystems in Antarctica are one of Earth's last, relatively intact, large marine ecosystems that lacks direct human impact (Chown, Clarke et al. 2015). For coastal marine species, certain groups such as pycnogonids show their highest richness in Antarctica and Antarctic biodiversity can be on par with shelf areas outside coral reefs in temperate and tropical waters (Chown 2012).

As stated above the principal objective of the current study was to assess the abundance and biomass, of marine invertebrates on hard substrata at Rothera Point and compare with its first survey in 1998. In the austral summer (November – March) of 1998 one of the first, and most detailed, quantitative studies of benthic faunal abundance, diversity and biomass at that time was carried out at Cheshire Island off Rothera Point, Adelaide Island, Antarctica. This chapter reports a very similar study now, carried out during the austral summer of 2015, and set in the context of observed changes in the benthic fauna at the same location and over roughly the same time period.

H1 will test the hypothesis, that there will be a change in proportion of mobile to sessile benthic species, there will be more mobile animals and less sessile animals in shallow rocky habitats around Rothera Point.

Barnes and Souster (2011) showed that the annual mortality of the sessile bryozoan *Fenestrulina rugula* had a correlation with the local frequency of ice scouring. Barnes (2016) reported decreases in the smaller and encrusting component of sessile fauna. H2 will test the hypothesis, that the abundant echinoid *Sterechinus neumayeri* will now be even more plentiful as it is often found in high abundance around scoured benthic areas and is mobile, therefore able to colonise newly barren benthos. Smale et al. (2007) showed *S. neumayeri* density increased with scouring and H3 will test the hypothesis that the overall mean faunal abundance and biomass will be reduced due to increased disturbance from ice scour.

3.2 Methods

Study Area

The sample site for the current work is situated between South Cove and Cheshire Island (Ryder Bay see Figure 3.1). The site was chosen for its bathymetric profile, dominance of hard substrata and being the site of the 1998 biodiversity survey. The hard substratum found at South Cove occurs widely throughout the WAP and is considered a typical shallow, dynamic and highly heterogeneous habitat. Cheshire Island was also one of the first sites on the WAP where biodiversity was surveyed in detail (see Barnes and Brockington 2003). Samples were collected adjacent to the British Antarctic Survey Research Station on Rothera Point at the South West end of Adelaide Island, WAP. The annual seawater temperature range varies between -1.8°C and +1.5°C (Barnes 2017a). The sea surface freezes to form fast ice for several months over winter each year. During summer months there is open water and scour by brash ice and icebergs.

Again as mentioned above the method used is a subset of the method presented in Chapter 2's biodiversity survey as the protocol for this study had to be the same as the study used for the previous survey of the same site (Barnes and Brockington 2003). However, the current study did not include the intertidal area and only sampled to 20m depth. Samples were collected between January and March 2015 (austral summer). Previous survey samples were collected January – February 1998 (austral summer). The sites were accessed by SCUBA from rigid inflatable boats. The study in 1998 employed fewer quadrat replicates than this study especially at 20m where there were only two replicate samples

The abundance and biomass (wet mass) of benthic taxa were measured along three transects running between Cheshire Island and The Wharf adjacent to Rothera Point (Figure 3.1). Three transects (denoted 1, 2 and 3 in Figure 3.1) were selected on hard bedrock substrata from preliminary dive surveys. The sample depths of 6m, 12m and 20m were chosen as

these depths are the same sampling depths as (Barnes and Brockington 2003). However, these are also the deepest depths which allow enough time for substantial in water work using the no decompression dive time allowed on DCEIM tables (~25 min) and to sample within two distinct zones.

Five randomly chosen quadrats (subset) from each depth (Figure 3.2) were used for the comparison between 1998 and 2015. Identification taxonomic levels were standardised with Barnes and Brockington (2003) to ensure comparability of biodiversity and biomass data from 1998 (see Table 3.1). For example, in the 1998 study asteroids and holothurians were not separated into different species, possibly due to limitations in identification. Comparisons were therefore made based on the lowest comparable taxonomic identification in 1998. The samples were collected using a 0.25m² quadrat and all organisms visible were removed by hand and placed in mesh bags before being returned to the laboratory to be sorted into morphospecies, ensuring the organisms were submerged in water at all times.

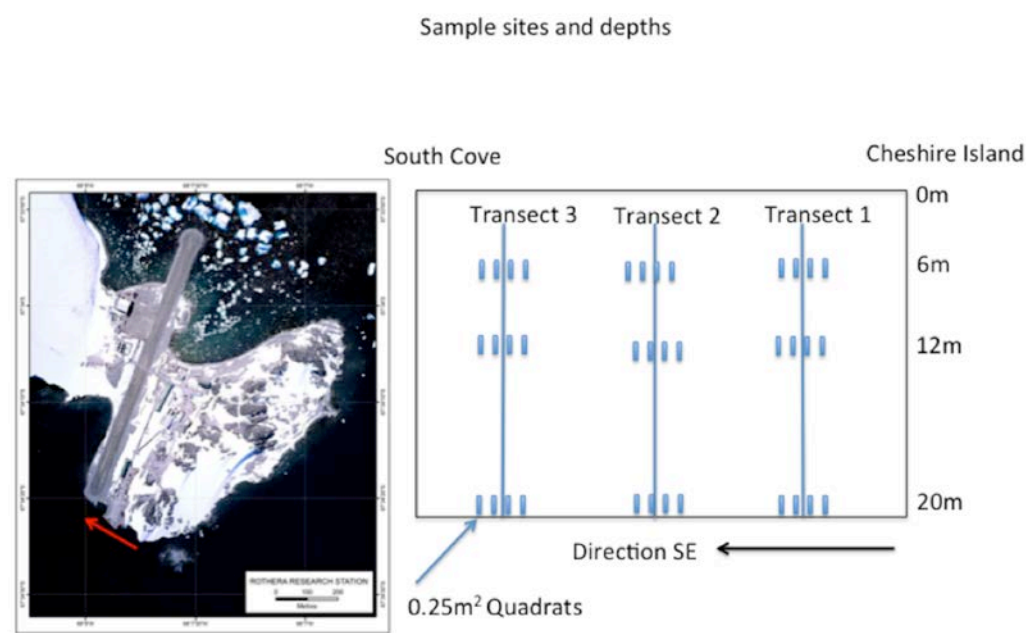


Figure 3.1 Aerial photograph of Rothera Research Station showing the sampling area (Red Arrow) and the three transects with three depths shown by the caption.

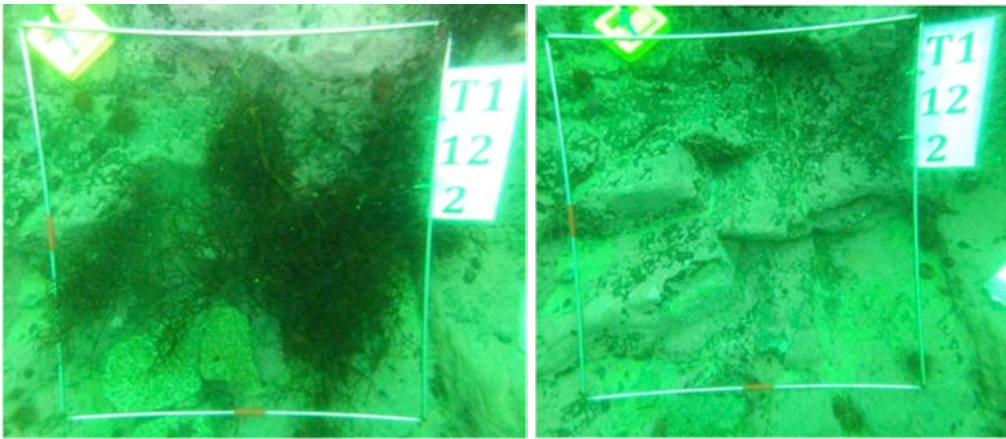


Figure 3.2 Example of a 0.25m² quadrat on transect one (T1) at 12m (12). The images are prior (left image) and after faunal sampling (right image).

Using primary literature and identification guides, individuals were identified to morphospecies level where possible (or preserved in 95% ethanol for subsequent identification). Organisms were blotted dry by placing them on kitchen roll and rotating three times, then placing them on a microbalance (Sartorius LA3200D 1mg accuracy) to obtain wet mass.

Statistical analysis

Replicate samples from each depth in each year were averaged to give macrofauna abundance, species richness and biomass m⁻². The data were collected within 0.25m² quadrats and then expressed as the mean number m⁻². Prior to analysis using a general linear model ANOVA, data were tested for normality using Anderson-Darling tests. The data were also tested for homogeneity of variance using Levene's test. Normally distributed, homogeneous data were analysed using GLM (ANOVA) performed using statistical analysis software (MINITAB) version 17 for Windows.

Simpsons Diversity Index was used as a quantitative measure. It reflects the number of different species in a sample but taking into account how evenly the individuals are distributed among those species. Simpson's Index (**D**) is a measure of diversity which takes into account both species richness and evenness of abundance among the species present. It estimates the probability that two individuals randomly selected from an area will belong to the same species. The value **D** for Simpson Index

ranges from 0 to 1, with 0 being infinite diversity and 1 representing no diversity.

Multivariate analysis of assemblage structure was performed using the Primer v. 7 statistical packages. Bray Curtis similarity matrices were produced based on the abundance and biomass data of the macrofauna, which were pre-treated with square root transformations to down weight, the influence of highly abundant or large taxa. The similarity of assemblages at each depth and each year was assessed using non metric multidimensional scaling (nMDS) and the clustering was tested statistically using SIMPROF groups, whilst the statistical significant difference between depths and years was tested using a crossed two way analysis of similarities (ANOSIM). SIMPER analysis was then used to test which species were responsible for the difference between depths and years.

3.3 Results

A total of 22 morphospecies representing 13 classes and 9 phyla were identified. Sampling numbers approached asymptotes suggesting appropriate sampling effort (Figure 3.3) and the samples are a good representation of the species richness within our study site.

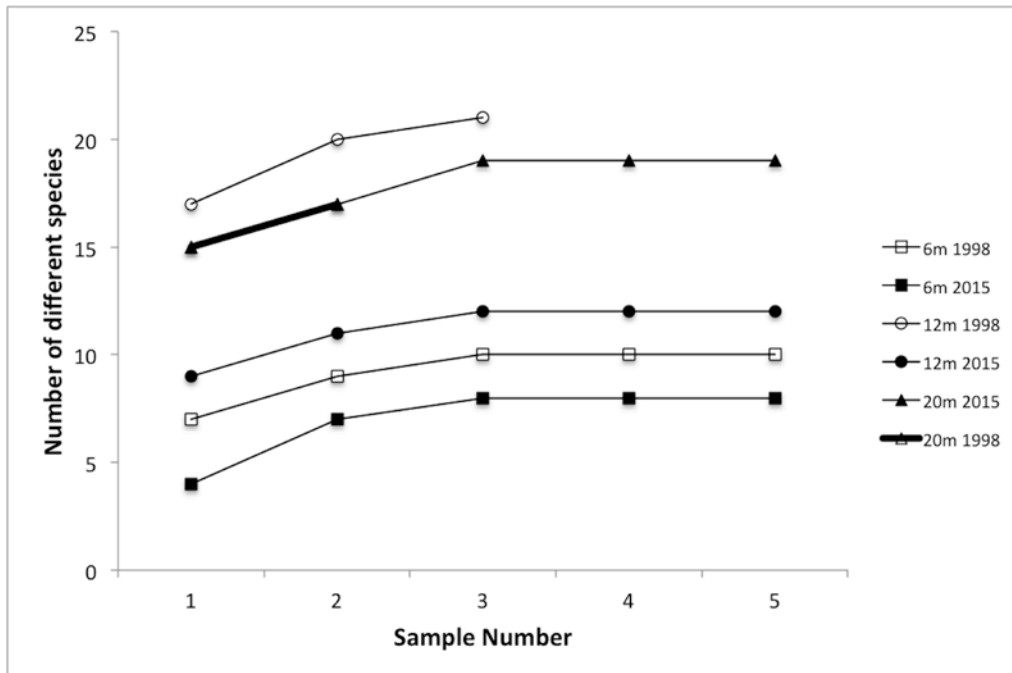


Figure 3.3 Species accumulation curve at each depth in both sampling years.

Phylum	Class	Order	Species	Vagile	Sessile
Mollusca	Gastropoda	Docoglossida	<i>Nacella concinna</i>	*	
			<i>Margarella antarctica</i>	*	
			<i>Iothia emarginuloides</i>	*	
		Nudibranchia	Nudibranchs	*	
	Polyplacophora		Chitons	*	
	Bivalvia		Bivalves		*
			<i>Limatula ovalis</i>		*
Echinodermata	Asteroidea	Phanerozonida	<i>Odontaster validus</i>	*	
			<i>Diplasterias brucei</i>	*	
			<i>Perknostaster</i> spp	*	
	Echinoidea	Echinoida	<i>Sterechinus neumayeri</i>	*	
	Holothuroidea	Dendrochirotida	Holothurians	*	
	Ophiuroidea	Ophiurida	<i>Ophionotus victoriae</i>	*	
			<i>Ophiura crassa</i>	*	
Nemertea	Anopla	Heteronemertea	Nemerteans	*	
Annelida	Polychaeta		Scale worms	*	
			Terebellids		*
Platyhelminthes			Flatworms	*	
Crustacea	Malacostraca	Amphipoda	<i>Paraceradocus miersii</i>	*	
Chelicerata	Pycnogonida	Pantopoda	Pycnogonids	*	
Cnidaria	Anthozoa	Alcyonacea	<i>Alcyonium antarcticum</i>		*
Chordata	Ascidiacea	Stolidobranchia	<i>Cnemidocarpa verrucosa</i>		*

Table3.1 Full morphospecies list used in the comparison of benthic biodiversity in 2015 with the same study area sampled in 1998.

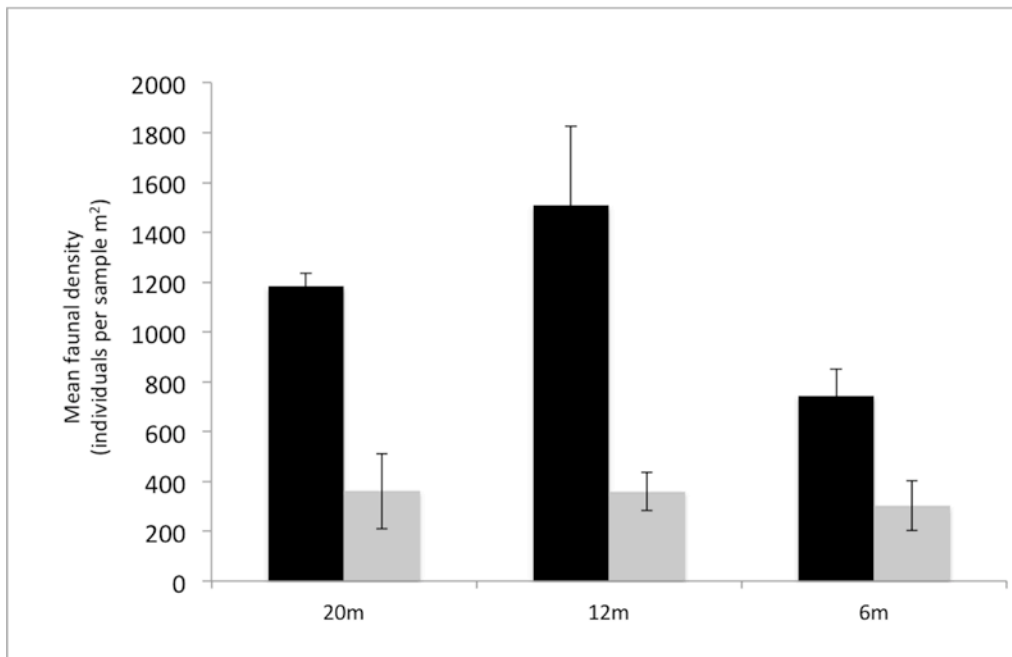


Figure 3.4 Mean faunal density at 6m, 12m and 20m in the austral summer of 1998 (Black bars) and the austral summer of 2015 (Grey bars) means \pm SD.

There was no significant difference between densities across depths (GLM $F_{(1,14)} = 0.42$, $P = 0.664$) in either year (Figure 3.4). There was, however, a significant difference in animal density of animals between 1998 and 2015 (GLM $F_{(1,19)} = 154.68$, $P < 0.001$). The density of animals at each depth was significantly greater in 1998 than 2015 (GLM $F_{(2,19)} = 12.48$, $P < 0.001$).

The most abundant species in the 2015 study was the Antarctic limpet *Nacella concinna* that reached densities of 248.8m^{-2} at 6m and 250.4m^{-2} at 12m. However, at 20m the most abundant species was the cushion star *Odontaster validus* with 74.4m^{-2} . The most abundant species in the 1998 study was the Antarctic urchin *Sterechinus neumayeri*, that reached a density of 495 ± 83 inds m^{-2} between 10m and 20m (Table 3.2). The largest difference in faunal density between 1998 and the current study was at 12m, in which faunal densities were 1508 ± 318 inds m^{-2} and 360 ± 78 inds m^{-2} respectively (Figure 3.4).

Most abundant species found in 2015 compared with 1998 (density m ⁻²)			
Depth (m)	Species	2015	1998
6	<i>N. concinna</i>	248.8	484.8
12	<i>N. concinna</i>	250.4	90.7
20	<i>O. validus</i>	74.4	30.0
Most abundant species found in 1998 compared with 2015 (density m ⁻²)			
6	<i>N. concinna</i>	248.8	484.8
12	<i>S. neumayeri</i>	23.2	493.3
20	<i>S. neumayeri</i>	28.8	498.0

Table 3.2 Table showing differences in the most abundant species between 1998 and 2015

The Simpsons Diversity Index showed that biological diversity was significantly higher ($D = 0.14$) in 1998 than 2015 ($D = 0.29$) at 12m. There was no significant difference between the diversity at 6m and 20m in 1998 compared with the current study (Figure 3.5).

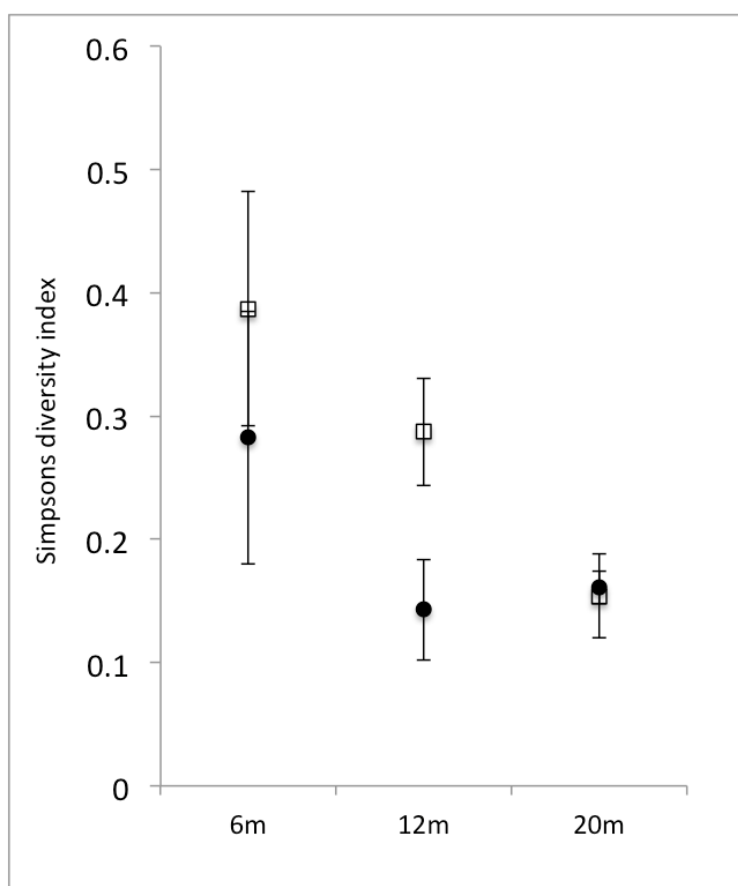


Figure 3.5 Species diversity of macrofauna at different depths in 1998 (•) 2015 (□). Only samples from 12m depth were significantly different between years (T test $P = 0.004$). Error bars are \pm SD.

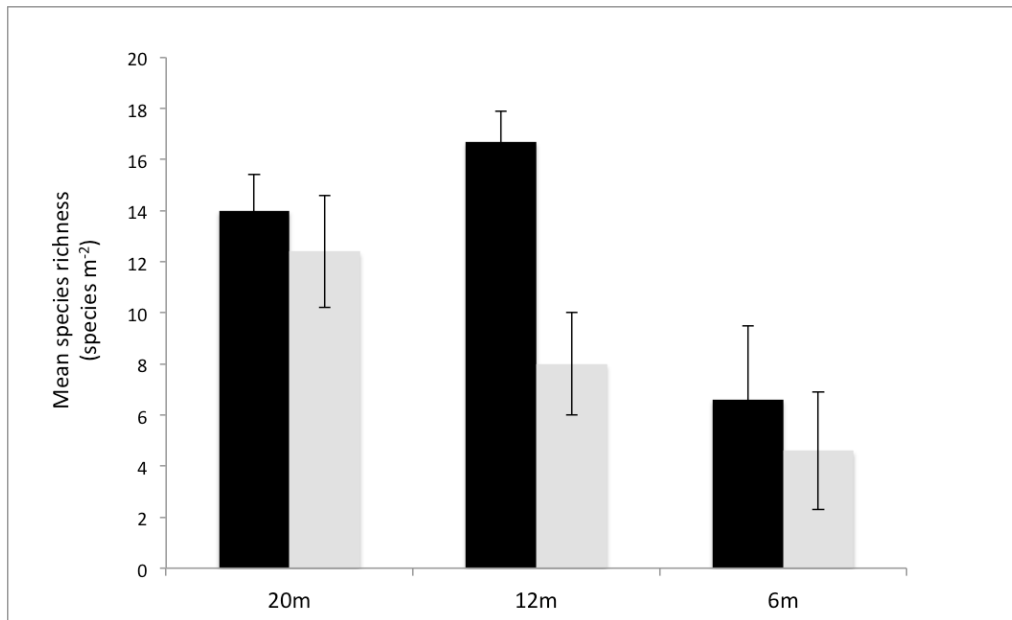


Figure 3.6 Mean species richness (+/- SD) at three depths in the austral summer of 1998 (black bars) and the austral summer of 2015 (grey bars).

Species richness was significantly different between both years (GLM $F_{(1,19)} = 18.57$, $P < 0.001$) and depths (GLM $F_{(2,19)} = 29.23$, $P < 0.001$). There was also a significant interaction between depth and year (GLM $F_{(2,19)} = 5.95$, $P = 0.01$). The largest difference in species richness was at 12m with 16.7 +/- 0.7 species documented in 1998 and only 8 +/- 0.9 species in 2015. The lowest species richness was at 6m in the 2015 study with a mean of just 4.6 species m⁻² (Figure 3.6).

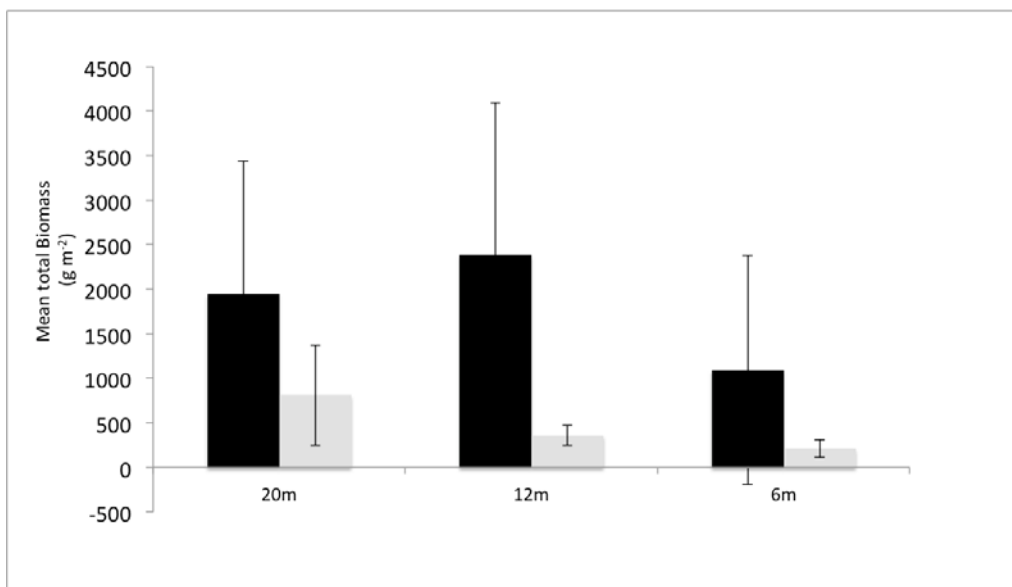


Figure 3.7 Mean biomass (+/- SD) at three depths in the austral summer of 1998 (black bars) and the austral summer of 2015 (grey bars).

There was a significant difference in biomass (wet mass g) between depths in both years (GLM $F_{(2,19)} = 5.08$, $P < 0.05$) and between years (GLM $F_{(1,19)} = 20.83$, $P < 0.001$; Figure 3.7). However there was no significant difference in the interaction of years with depth (GLM $F_{(2,19)} = 0.61$, $P = 0.553$). Biomass was much higher in 1998 at all three depths compared with the current study. The largest biomass was 12m in 1998, with a total biomass of 2,387g. The current study on the other hand had 360.6g at 12m.

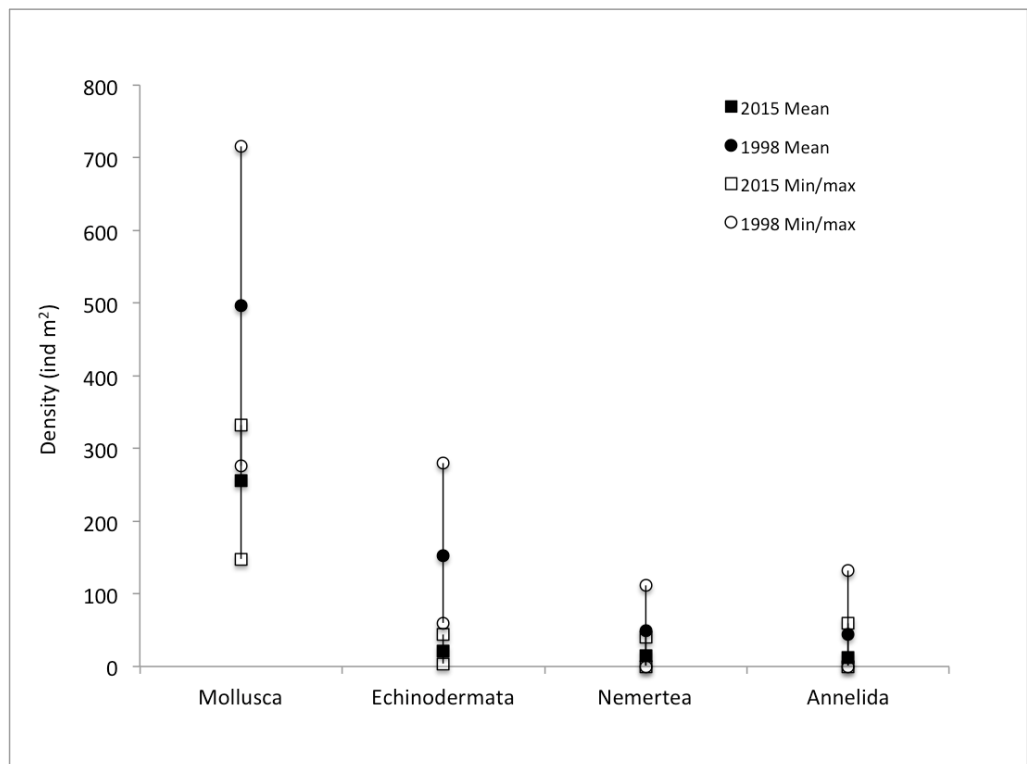


Figure 3.8 Comparison of faunal abundance across four different phyla at 6m in the austral summer of 1998 with the austral summer of 2015.

There were no significant differences in density for the four most common phyla at 6m between years (Figure 3.8, variation is expressed as 95% confidence intervals).

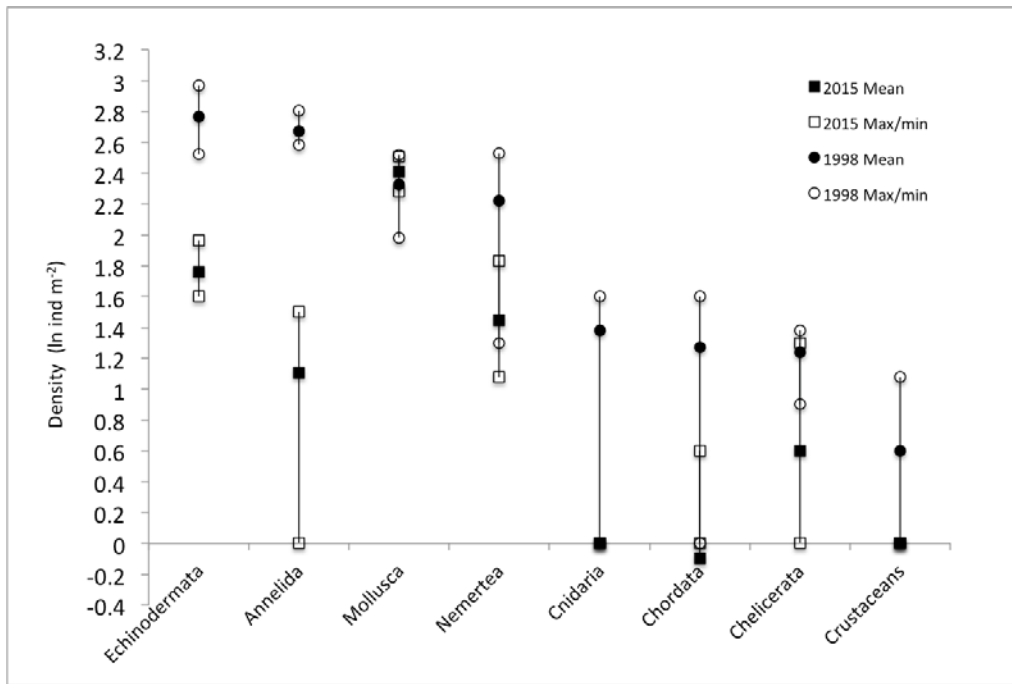


Figure 3.9 Faunal density (log ln transformed) with phylum at 12m in the austral summers of 1998 and 2015.

The densities of two phyla, Annelida and Echinodermata were different between 1998 and 2015 at both 12m and 20m (Figure 3.9 and 3.10 respectively, variation is expressed as 95% confidence intervals). The total number of Echinodermata in 1998 was significantly higher than in 2015 (ANOVA $F_{(1,6)} = 16.49$, $P = 0.007$). The density of Annelida was also significantly higher in 1998 compared with 2015 (ANOVA $F_{(1,6)} = 56.33$, $P < 0.001$), whereas all other phyla (which were log transformed) were not significantly different with time (ANOVA $P > 0.05$).

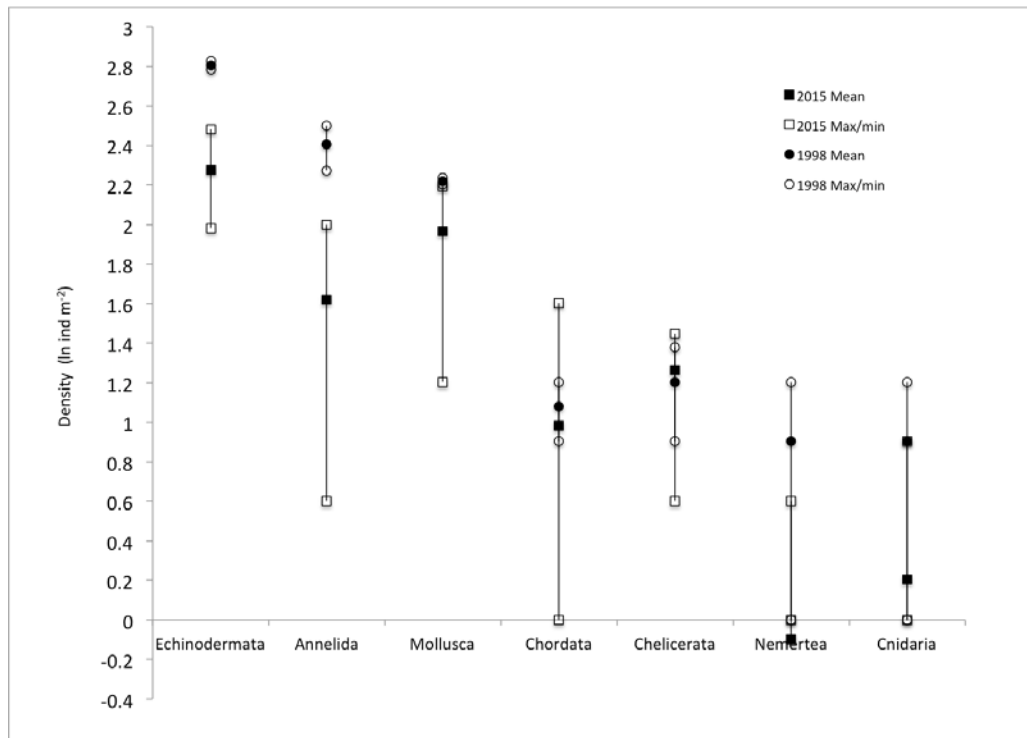


Figure 3.10 Faunal density (log ln transformed) with phylum at 20m in the austral summers of 1998 with 2015.

Two taxonomic groups, Echinodermata and Annelida were significantly different in density between years at 20m (Figure 3.10, variation is expressed as confidence 95% intervals). There was a significantly higher density of Annelida in 1998 compared with in 2015 (ANOVA $F_{(1,5)} = 21.31$, $P = 0.006$), there was also far more variability in Annelida density data in 2015 with the lowest density being 4 inds m^{-2} and the highest being 100 inds m^{-2} . There was a significantly higher density of Echinodermata in 1998 compared with 2015, with mean densities of 640 inds m^{-2} and 188.8 inds m^{-2} respectively (ANOVA $F_{(1,5)} = 40.38$, $P = 0.001$). The main species contributing to the large difference in echinoderm density between 1998 and 2015 was *S. neumayeri* with mean densities of 498 inds m^2 and 28.8 inds m^{-2} respectively.

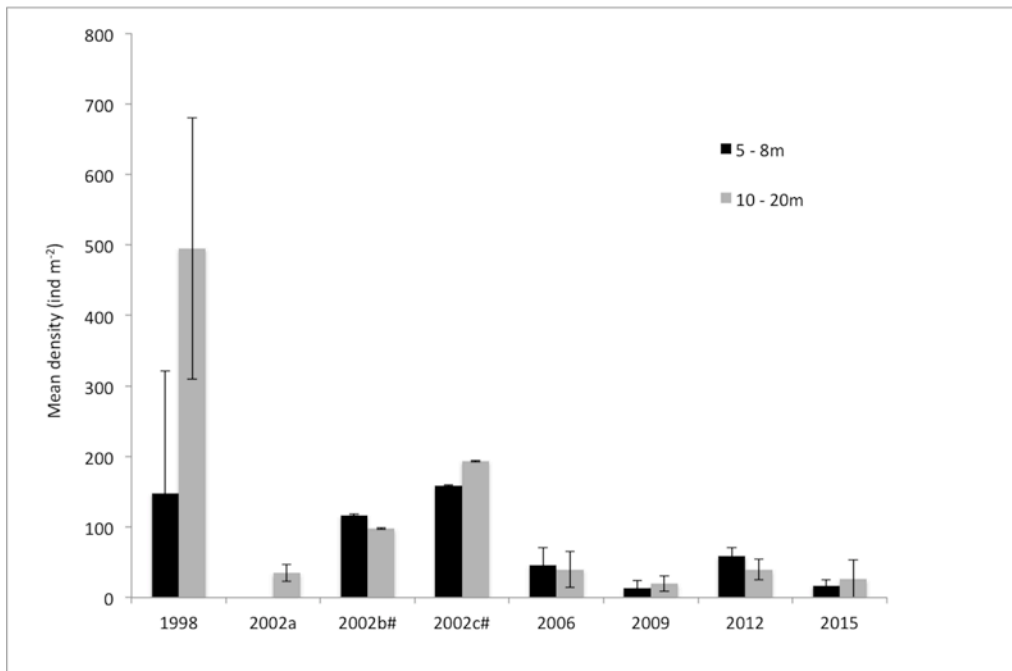


Figure 3.11 Comparison of *S. neumayeri* density with year from 1998 until the current study in 2015. Values are mean \pm 95% CI, N = number of quadrats. # indicates estimates of assemblages at both South Cove and Anchorage combined, all other data are from South Cove or Cheshire Island (Figure 3.1). 1998 Brockington et al. (2003) N = 5, 2002a Brown et al. (2004) N = 212, 2002b/c Bowden (2005) N = 28, 2006 Smale (unpublished) N = 24, 2009 Barnes (unpublished) N = 25, 2012 Cordingley (unpublished) N at 5 – 8m = 25, N at 10 – 20m = 50 and 2015 (Current study) N at 6 – 8m = 5, N at 10 – 20m = 10.

Changes in the density of *S. neumayeri* were a major part of the overall differences between 1998 and 2015 in faunal density and faunal biomass (Figures 3.4 and 3.7 respectively). The most abundant species in the study in 1998 was the Antarctic urchin *S. neumayeri* that reached a density of 495 ± 83 inds m^{-2} between 10m and 20m. Figure 3.11 shows *S. neumayeri* density data from several surveys at Rothera Research Station since 1998. There was no significant difference between the *S. neumayeri* abundance data in 1998 compared with the other years based on 95% confidence intervals. Nor was there a significant difference in *S. neumayeri* abundance data in 2006, 2009, 2012 and 2015. There was a significant difference in *S. neumayeri* abundance between 2002b and 2002c, however these data points are assemblage means at both South Cove and Anchorage Island combined and Anchorage Island is over 3km from Rothera Point.

		Biomass (Wet Mass) g					
		20m depth		12m depth		6m depth	
		1998	2015	1998	2015	1998	2015
Vagile Fauna	Asteroids	201.6	146.0	146.8	121.8	25.7	21.3
	Holothurians	751.2	159.7	939.1	0.0	0.0	0.0
	<i>Sterechinus neumayeri</i> *	347.1	79.0	370.0	76.0	587.1	114.3
	<i>Nacella concinna</i>	18.2	57.4	75.4	117.7	376.2	65.8
Sessile Fauna *							
	<i>Cnemidocarpa verrucosa</i>	59.6	9.6	118.3	1.5	0.0	0.0
	<i>Alcyonium antarcticum</i>	16.1	1.6	74.1	0.0	0.0	0.0
	Terebellids	493.1	13.6	567.3	1.8	52.5	2.8

Table 3.3 Comparison of the biomass (wet mass) in 1998 with 2015 in both vagile and sessile fauna. * Indicates a significant difference between years. Taxonomic levels used are identical to those of the 1998 survey.

Of the vagile fauna, *S. neumayeri* biomass (wet mass) was significantly different between 1998 and 2015 (One way ANOVA $F_{(1,21)} = 6.75$, $P = 0.017$) but there was no significant difference in biomass between the depths 6m, 12m and 20m in either year (One way ANOVA $F_{(2,21)} = 0.36$, $P > 0.05$). There was no significant difference in biomass of holothurians, asteroids or *N. concinna* between 1998 and 2015. Biomass of all the sessile fauna was significantly lower in 2015 than 1998 (One way ANOVA $F_{(1,21)} = 8.83$, $P = 0.007$) and significantly different between depths (One way ANOVA $F_{(2,21)} = 4.36$, $P = 0.026$).

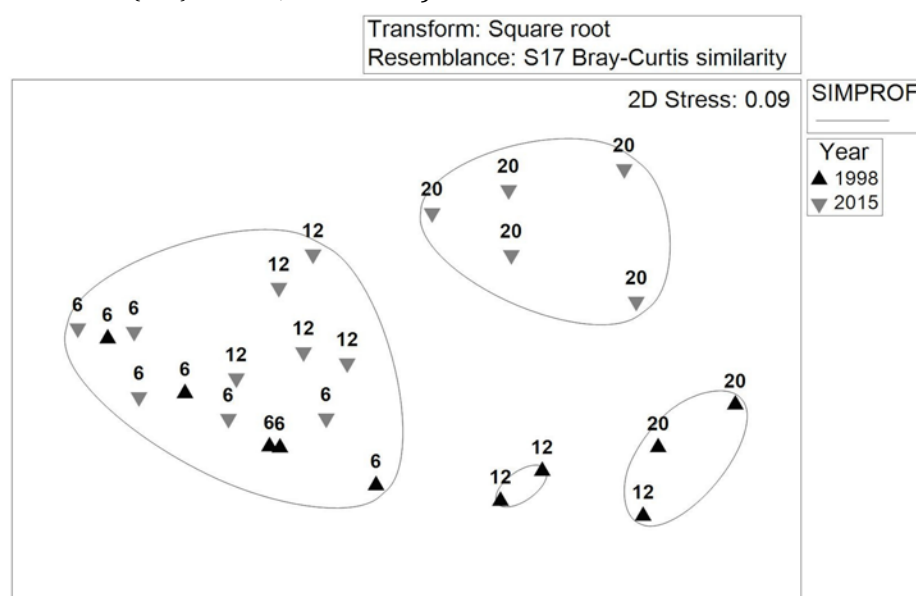


Figure 3.12 nMDS ordination of faunal density data collected from three depths in 1998 and 2015 on square root transformed abundances and Bray Curtis similarities (stress = 0.09). Four significantly different ($P < 0.05$) groups were identified, using SIMPROF after 9999 permutations and a cophenetic correlation $\rho = 0.81$.

The nMDS ordination of samples at the three study depths shows distinct clustering and some gradient separation by year and depth, with 1998 and 2015 being grouped differently (Figure 3.12). In 1998, the 12m and 20m samples were also closely clustered which was also seen in the faunal density (Figure 3.4), species richness (Figure 3.5) and faunal biomass (Figure 3.6). ANOSIM $R = 0.6$ shows a good separation of the years, which is significant at $P < 0.001$. The results from the SIMPER analysis showed that the taxa most responsible for the observed differences between 1998 and 2015 were *S. neumayeri* followed by *N. concinna* and then terebellid polychaete (Table 3.3). The nMDS ordination bubbleplot of *S. neumayeri*, *N. concinna* and terebellid polychaete density shows how their density influences this grouping in different depths and years (Figure 3.13).

1998 versus 2015 (ANOSIM $R = 0.6$, $P < 0.001$) SIMPER Average dissimilarity = 39.38				
Species	Average abundance		Average Dissimilarity	% contribution
	1998	2015		
<i>S. neumayeri</i>	13.00	4.38	6.79	17.24
<i>N. concinna</i>	7.22	12.85	6.78	17.22
Terebellids	8.44	1.82	5.14	13.05

Table 3.4 Results from a two way crossed ANOSIM and SIMPER on square root transformed average density data showing the taxa contributing to 50% of the difference between 1998 and 2015.

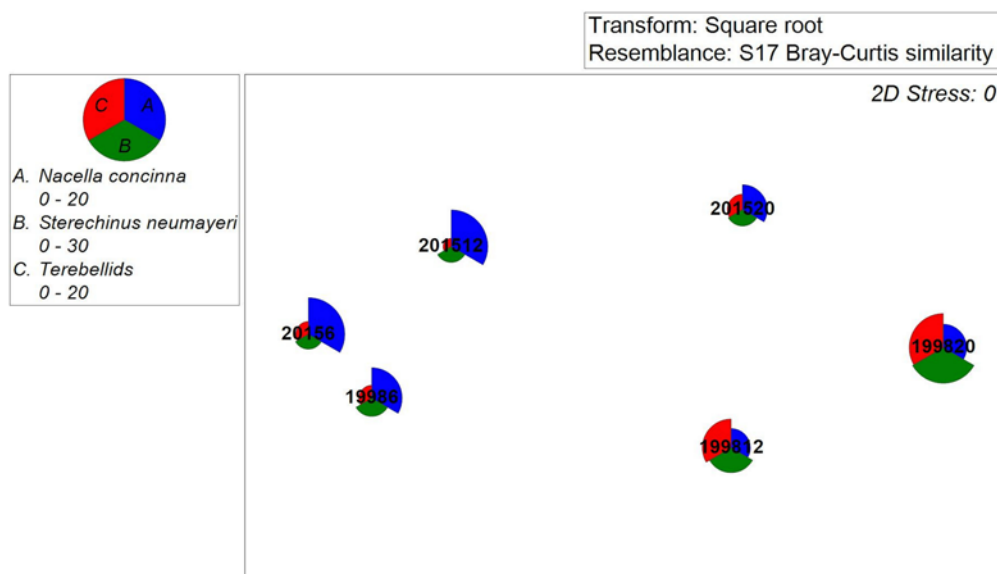


Figure 3.13 nMDS bubble plot of the three taxa with most contribution to the differences in groups, using square root transformed average abundance at the three depths in both 1998 and 2015.

The nMDS values for faunal biomass in 1998 and 2015 were generally well separated by year and depth (Figure 3.14) and an ANOSIM R value of 0.5 with $P < 0.001$. The SIMPER analysis identifies the organisms contributing most to the difference in biomass between years (Table 3.3) and in this study the top five taxa are terebellid polychaetes, *S. neumayeri*, holothurians, scale worms and *N. concinna* respectively. Figure 3.15 shows a reduction in terebellid polychaete biomass at three depths from 1998 to 2015.

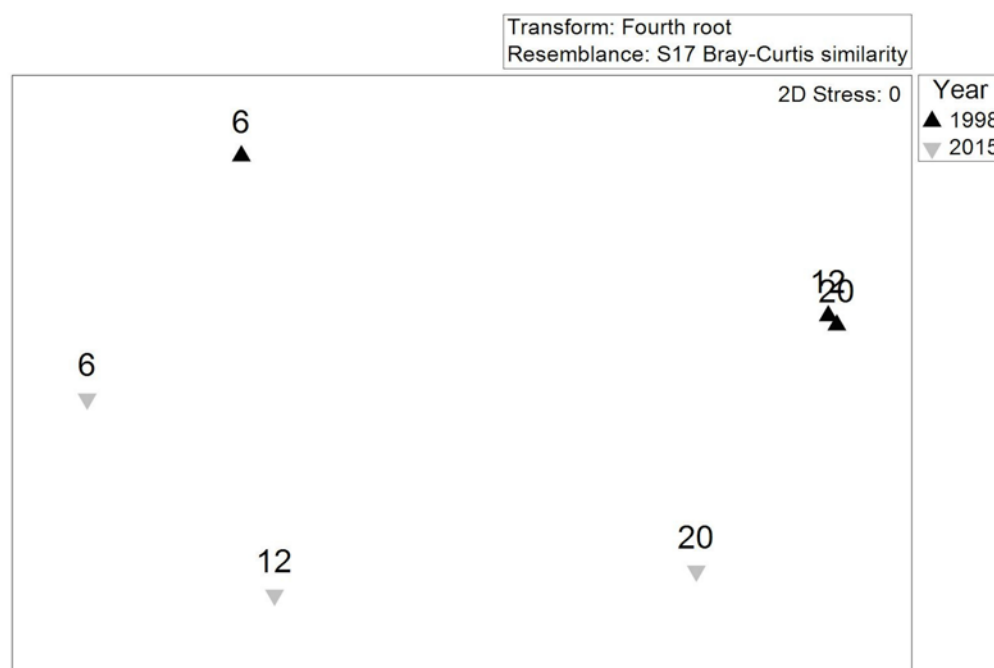


Figure 3.14 nMDS ordination of average biomass (Wet Mass) data collected from three depths in 1998 and 2015 on fourth root transformed and Bray Curtis similarities (stress = 0).

1998 versus 2015 (ANOSIM $R = 0.5$, $P < 0.001$)				
SIMPER Average dissimilarity = 37.02				
Species	Average Biomass (g)		Average Dissimilarity	% contribution
	1998	2015		
Terebellids	3.62	0.94	5.49	14.82
<i>Sterechinus neumayeri</i>	4.37	2.67	3.92	10.58
Holothurians	3.06	0.98	3.86	10.41
Scale Worms	2.19	1.04	2.89	7.82
<i>Nacella concinna</i>	3.00	2.93	2.27	6.13

Table 3.5 Results from a two way crossed ANOSIM and SIMPER on fourth root transformed biomass data showing the five types of animal contributing to 50% of the difference between years.

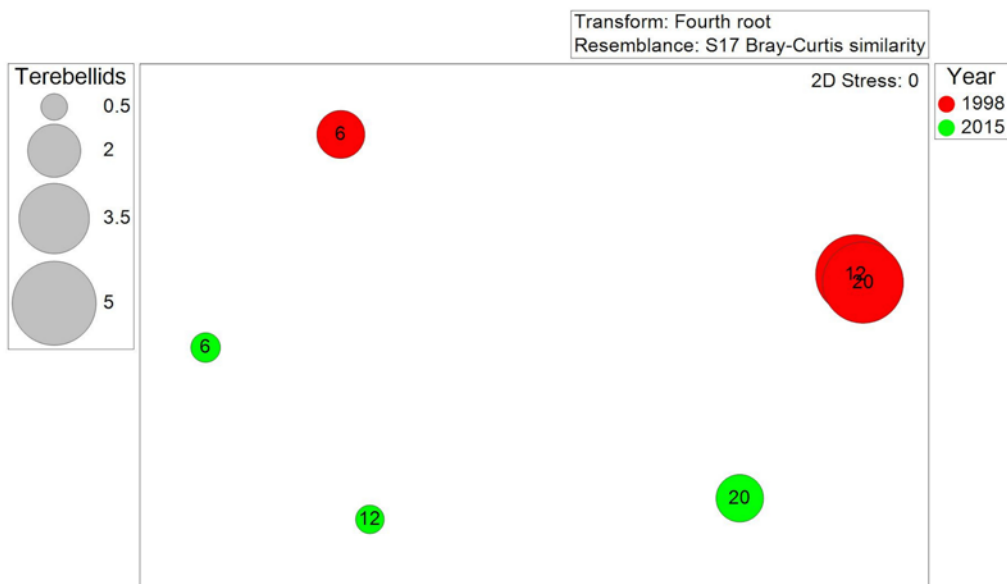


Figure 3.15 nMDS bubble plot of average biomass (Wet Mass) of terebellids at three depths in 1998 and 2015.

In summary, the greatest change between 1998 and 2015 was at 12m.

There were significant differences: in faunal density (GLM $F_{(1,19)} = 154.68$, $P < 0.001$), biological diversity (T Test $P = 0.004$), species richness (GLM $F_{(1,19)} = 18.57$, $P < 0.001$) and faunal biomass (GLM $F_{(1,19)} = 20.83$, $P < 0.001$) between years.

3.4 Discussion

The research project reported here was successful in that it addressed the questions set out in the introduction, to compare the biodiversity of the same area at two different points in time. However, it is still only based on two time points. This study would have been enhanced by a further biodiversity and biomass study carried out in an additional year and also a study from an undisturbed site. The study carried out in 1998 had far fewer quadrat replicates than this study, especially at 20m where there were only 2 replicate samples, which restricted the power of the comparisons.

While there were similarities between the years the main difference in biodiversity and biomass was found at 12m where there were at least three replicates. The 1998 study was the first comprehensive shallow benthic biodiversity study on the Antarctic Peninsula and there were limitations to the taxonomic levels to which organisms were identified. However this did not seem to have a large impact on the outcome, as the species which contributed most to the changes (*N. concinna* and *S. neumayeri*) were all identified to species level. Also, since 1998 we now know there is only one species of terebellid worm large enough to be collected using the method in 1998 in the coastal shallows at Rothera; *Thelepus cincinatus* (Souster and Clark pers obs).

Shallow water and coastal environments of the Arctic and West Antarctic are changing rapidly (Barnes 2017a). Here we show a decrease of biodiversity, faunal density, species richness and biomass from 1998 to 2015 and from this we might infer reductions in the coastal shallows of both biodiversity and organic biomass at sites near Rothera Research Station. There has been a large reduction in organic biomass especially at 12m due to ice scour effects particularly on terebellids. Smale et al. (2007) found that at sites near the present study compared with undisturbed areas, scour assemblages were 95% lower in mean macrofaunal abundance and 75.9% lower in species richness. Ecosystems with lower biological diversity are less resilient to disturbance than biologically rich

areas (Chapin, Zavaleta et al. 2000) and the IPCC predict show that the global glacier volume, is projected to decrease by 15-55% by the end of the century meaning increasing ice shelf calving and the number of icebergs present in the Southern Ocean. The IPCC predicts increased shallow water disturbance events with climate related extremes.

We hypothesised that there would be an increased abundance of the echinoid *S. neumayeri* in the shallows due to increased ice scouring (H1). Smale et al. (2007) previously showed that *S. neumayeri* density increased with scouring. The current study did not support this finding; there was no significant difference in the population of *S. neumayeri* in the shallows in 2015 compared with 1998 (Figure 3.11 95% confidence intervals overlap). However, there was a significant difference in *S. neumayeri* biomass (wet mass). This could be interpreted as a change in the size frequency of the *S. neumayeri* population in Ryder Bay. The mean urchin wet mass (g) for *S. neumayeri* in the 2015 study their wet mass was four times higher than in 1998, lower density of *S. neumayeri* but higher mean urchin wet mass (g).

Ideally a longer time series would be used for the comparison of change in abundance and biomass in the urchin *S. neumayeri* would be carried out using three data points instead of just the two (1998 and 2015), however there are multiple studies since 1998 which looked at *S. neumayeri* density (Figure 3.11) and these show a similar result to 2015.

S. neumayeri is the most abundant sea urchin in shallow Antarctic waters, with a circum-Antarctic and sub-Antarctic (to Kerguelen Island) distribution, and extending from the shore down to about 400m water depth (Brey and Gutt 1991). There are a few potential explanations for a change in size of *S. neumayeri*. These include firstly, a change in light levels affecting biofilm levels in the shallows. The Antarctic urchin is a generalist omnivore that feeds mainly on diatoms and algae that are seasonal (Brockington, Clarke et al. 2001). Antarctic biofilms exhibit large variability in community structure and biomass over short time scales

(Gilbert 1991, Rochera, Fernández-Valiente et al. 2013). *N. concinna* is another abundant and often dominant macrobenthic invertebrate in the coastal Antarctic shallows, and the most abundant invertebrate at 6m and 12m in this study (Table 3.2). *N. concinna* increases in size with depth, and shows changes in shell shape, consistent with ice disturbance effects (Hoffman, Peck et al. 2009). Secondly, the frequency of ice scour reduces with depth; at 25m ice scour frequency is 1/3 of that recorded at 5m (Barnes 2017a). Thirdly, it is not only ice scour, which makes polar continental shelves 'extreme' environments. Antarctic benthos in the shallows are also subject to other devastating impacts including anchor ice and high wave action (Barnes and Conlan 2007). Storm force wind and waves can have a pronounced influence on shallow and intertidal communities at all latitudes, such that single events can affect their community structure for years afterward (Wulff 1995). The limpet *N. concinna* (due to its tenacity) is more likely to be able to withstand the force of strong waves compared with *S. neumayeri*. Storm force winds are able to influence biodiversity down to at least 14m depth (Peck, Brockington et al. 1999) when 148kmh⁻¹ wind speeds created water movements powerful enough to redistribute small macrofauna such as the bivalve *Mysella charcoti* at a previously ice scoured site.

This study compared biodiversity and biomass at 6m, 12m and 20m but it was sampling at 12m that showed the greatest change since 1998. At 12m there was a significant difference between 1998 and 2015 in faunal density, diversity, richness and biomass ($P < 0.005$). Barnes and Souster (2011) showed that the duration of fast ice at Rothera had decreased significantly by $> 5\text{d yr}^{-1}$ over the last 25 years and that this was strongly correlated with increased ice scour and mortality of benthos in the shallows. Ice scour frequency varied with depth but in that study increased most significantly at 10m compared to 5m or 25m, which could explain the greatest change found in this study (at 12m). At 10m, Barnes and Souster (2011) reported that the annual mortality of *Fenestrulina rugula* showed a highly significant correlation with the local frequency of ice scouring. At 10m, annual scour probability per m² of seabed was 25%,

the mean time since last scour was 3 years and only 18.7% of the sea bed was not scoured within 5 years (Barnes 2017a).

In the Canadian Arctic, Conlan and Kvitek (2005) followed faunal recovery of 19 iceberg scours and suggested that >10 yrs. is required for scoured communities to recover to background levels. Barnes (2016) suggested the time scale for recovery is very similar in Antarctic recovery, at least in terms of benthic carbon stocks. However Smale et al. (2007) found so much variation between scour recovery durations that determination of a set time was not considered meaningful. With only 18.7% of seabed not scoured at 10m within 5 years (Barnes 2017a) a 10 year recovery period for communities could be the reason for a large decline in faunal abundance and biomass at 12m between 1998 and 2015. Thus we suggest that the third hypothesis (H3), that overall benthic faunal abundance and biomass will be reduced due to increased ice scour, is accepted as the patterns of decrease in abundance and biomass most closely matched the effects of increased ice scour as the greatest change occurred at 12m which coincided with the greatest increase in ice scour.

In the current study the shallow water fauna comprised of many sessile animals and those ectotherms, which do move, do so slowly (Chapter 4). It is likely that few except fish and amphipods can move out of the way to avoid agents of catastrophic local disturbance such as icebergs (Gutt, Starmans et al. 1996, Peck, Brockington et al. 1999, Gutt 2001). Sublittoral sessile epifaunal assemblages are often characterized by similar groups of organisms. Bryozoans, calcareous polychaetes, sponges, ascidians, and hydrozoans are common components of hard substratum sessile assemblages at all latitudes (Bowden, Clarke et al. 2006).

In situ photographs and videos demonstrate that iceberg grounding in both polar regions causes considerable damage to benthic communities. Sessile organisms on hard substrata are eradicated (at small spatiotemporal scales) and pioneer species recolonise in high abundances on the newly available substratum (Gutt, Starmans et al. 1996). Biomass of

all sessile fauna measured by this study was significantly less in 2015 compared with that reported in 1998 (ANOVA $F_{(1,21)} = 8.83$, $P = 0.007$). The group contributing most to this large decrease in biomass (also shown in SIMPER analysis) was the terebellid worm *Thelepus cincinatus* which could be extremely vulnerable to disturbance by ice scour (Table 3.3, Figure 13.5). Annelids such as *Thelepus cincinatus* represent a large amount of organic biomass (Chapter 2) that is then released back into the water column.

Mortality in encrusting organisms (e.g. bryozoans) due to ice scour could also explain part of the large biomass reduction as some of these are dominant sessile organisms (Barnes 2017a). Our hypothesis (H1) that there will be more mobile and less sessile animals in the coastal shallows has been partly supported in this study. Both groups showed declines; however abundance of sessile organisms declined more than mobile organisms, the most abundant animals were mobile and the greatest biomass loss was of sessile invertebrates.

Many shallow water polar communities are held at early successional stages by chronic ice scour (Dayton, Robilliard et al. 1974, McCook and Chapman 1993, Barnes 1995, Pugh and Davenport 1997) and these ice scour effects occur from the intertidal to depths around 600m in Antarctica (Harris and O'Brien 1996, Peck, Brockington et al. 1999). The deepest palaeo-iceberg scours recorded to date are from the Arctic at a depth of 1200m (Arndt, Niessen et al. 2014). In conjunction with disturbance, the effects of large scale grazing at shallow depths may also drive the observed species distributions. In the shallows there are abundant *N. concinna* and *S. neumayeri* which are grazers. Bowden et al. (2006) reported strong effects of grazing at 8m depth on artificial hard substrata, which he suggested restricted the recruitment and development of sessile fauna. Frequently scoured areas may not recover, especially in the Antarctic due to slow growth rates of the fauna (Gutt 2001, Chapter 4) and slow larval recruitment, with colonization rates being up to 3 times

slower than comparable temperate latitude assemblages and predation from mobile grazing fauna (Bowden, Clarke et al. 2006).

3.5 Conclusion

Surface waters around the WAP have changed rapidly over the last 5 years. Increased air temperatures (Smith and Stammerjohn 1996), increased water temperatures (Meredith and King 2005), wind speeds and precipitation (Fox and Cooper 1998) all contribute to the loss of sea ice (Murphy, Clarke et al. 1995, Barnes 2017a), ice shelf collapse (Rignot, Bamber et al. 2008) and glacial retreat (Smith, Vaughan et al. 1999). Ongoing climate change in the coming decades is likely to drive more ice scour at the poles – through both reduced fast ice allowing more movement of existing icebergs and retreating glaciers producing more icebergs (Barnes 2017a). The impacts of less fast ice and more ice scour, along with other climate – forced changes are altering biodiversity and ecosystem processes and could change the resilience of ecosystems to environmental change (Chapin, Zavaleta et al. 2000). Predictions from the IPCC are for sustained and increased climate change in this region therefore it is likely that within the next century there will be even less biodiversity and biomass in the coastal shallows of the WAP with the most notable change being loss of organic biomass from sessile organisms that are destroyed by ice scour and then unable to re-establish themselves due to grazing pressure and increased frequency of disturbance. WAP ice scouring may be recycling 80,000 tonnes of carbon yr⁻¹ (Barnes 2017a). Loss of carbon biomass will also mean less carbon draw down and less removal of this carbon from the atmosphere that provides a negative feedback to climate change.

The greatest change between 1998 and 2015 was at 12m which coincides with the greatest increase in ice scour found to be at 10m (Barnes 2017a). Overall mean faunal abundance and biomass is less than was reported in 1998. Benthic biodiversity in the coastal shallows of Antarctica is changing there is a loss of sessile organisms such as the terebellid worm (Tables 3.4

and 3.5) and bryozoans (Barnes and Souster 2011). There is ongoing debate into the future of shallow water benthic ecosystems. Ice scour will continue to be a key driving disturbance force for benthic marine communities in Antarctica in the short term until all glaciers have reached their grounding lines and ice berg production is reduced or halted which may then given benthic communities the time to recover providing there are healthy communities in adjacent areas which will facilitate recolonisation efforts.

Chapter 4 – Seasonality of oxygen
consumption in five common benthic
marine invertebrates in shallow
rocky habitats, Antarctica

Chapter 4 Seasonality of oxygen consumption by five common benthic marine invertebrates in shallow rocky habitats, Antarctica

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4.1 Introduction

Routine and basal metabolic rate in marine ectotherms increases with temperature (Clarke and Johnston 1999, Clarke and Fraser 2004, Watson, Morley et al. 2013). Krogh (1916) noted that polar species are active at low temperatures and that temperate species are inactive when cooled, so thought polar species must have raised metabolic rates to allow activity. Early Antarctic studies in the 1950s and 1960s produced data that supported this idea (Wohlschlag 1964) and the concept of metabolic cold adaptation (MCA) was proposed. There have been many investigations of rates of oxygen consumption by polar marine ectotherms over the last 3 - 4 decades. The vast majority have shown metabolic rates in high latitude species to be much lower than in similar temperate species (Clarke and Peck 1991, Clarke and Johnston 1999, Peck and Conway 2000, Peck 2016). There have also been a few macrophysiology analyses of oxygen consumption across latitudes in phylogenetically controlled studies. None have found evidence supporting MCA (Clarke and Johnston 1999, Peck and Conway 2000, Peck 2016). The lowered metabolic rate of these species has been suggested to be a consequence of reduced basal costs at low environmental temperatures, of which protein turnover appears to be a major component (Clarke 1988, Fraser, Clarke et al. 2007, Peck 2016). Antarctic marine benthic invertebrates live at low but stable temperatures with an annual temperature range of between -1.8 and + 1.5°C (Barnes 2017a). A low standard or routine metabolic rate is the norm for polar marine ectotherms (Chapelle and Peck 1999, Peck 2002, Peck 2016). There is little or no evidence for compensation of rates in respect to

temperature, the metabolic rates are slowed by the cold rather than compensated (Peck 2016).

A large percentage of the biomass in shallow rocky habitats in Antarctica is made up of the common urchin *Stereochinus neumayeri*, the widely distributed sea star *Odontaster validus*, the widespread holothurian *Heterocucumis steineni*, the abundant limpet *Nacella concinna* and the common ophiuroid *Ophionotus victoriae*. In previous chapters the biomass in shallow rocky habitats was described and also compared in time, space and depth. This biomass is maintained and supported by the metabolism of the animals involved. Metabolism is the sum of all the aerobic processes within an animal. These are the processes that provide the energy for all biological functions (Clarke 2008), including the laying down of biomass, which in its simplest terms, is the growth of tissues and skeleton. To lay down biomass animals need to acquire resources by feeding, process the food, assimilate the absorbed molecules and lay down assimilated material as structure. Metabolic rate is an excellent predictor of physiological condition (Wilmer, Stone et al. 2000) in organisms.

Energy use is difficult to measure directly, as this requires the quantification of heat produced by the organism's metabolic processes, which is particularly difficult to detect in ectotherms. Metabolic rate is accurately estimated however, from oxygen consumption in fully aerobic conditions. Therefore in most whole animal investigations, oxygen consumption is used to estimate energy use (Clarke and Johnston 1999, Peck and Barnes 2004, Seibel and Drazen 2007). Consumption of oxygen is always accompanied by the production of CO₂, and therefore the rate of CO₂ production could also be used to measure metabolic rate. In marine species however, the assessment of CO₂ production is more difficult than the measurement of oxygen because evaluations must be determined with the animal submerged in seawater, which is complex with regard to CO₂ chemistry. Respired CO₂ interacts with the large carbonate buffer in seawater, making the quantification of the amount of CO₂ produced, if not impossible, then very difficult and with very large associated errors when using any standard techniques. So for marine species measurement of

oxygen consumption is the predominant method used to investigate metabolic rates.

When conducting experiments, the amount of dissolved oxygen is usually maintained above a level that might affect the oxygen consumption of the specimen under investigation (usually Oxygen above 60-70% saturation). Also, the measurement of oxygen utilization is usually only carried out for relatively short periods (Millman 1964) unless respiration rates are very low (Obermüller, Morley et al. 2010). Either that or specific methods are employed to allow long term trials, such as steady state through flow or stop flow systems (Behrens, Præbel et al. 2006, Brown, Heilmayer et al. 2010, Svendsen, Bushnell et al. 2016).

Although temperatures are stable in Antarctica, other factors including photoperiod and food availability vary markedly in polar regions, and more than at lower latitudes (Clarke 1988, Clarke, Meredith et al. 2008). Many Antarctic benthic marine invertebrates exhibit seasonal cycles in activities such as feeding (Barnes and Clarke 1995), growth (Barnes 1995, Kock and Everson 1998, Stanwell-Smith, Peck et al. 1999, Fraser, Clarke et al. 2002), storage of reserves (Clarke and Peck 1991) and reproduction (Pearse, McClintock et al. 1991, Grange, Tyler et al. 2004, Bowden 2005). Metabolic rates would therefore be predicted to vary with season. Metabolic rates vary with temperature as well as with the availability of food and these two variables may not necessarily change at the same time. For example, the sea temperature begins to warm in November during the austral summer, but the arrival of the phytoplankton bloom may vary depending on the previous winter fast ice break out (Riaux-Gobin, Poulin et al. 2011). However the seasonality of food supply seems to drive growth and reproduction, which will in turn have an effect on metabolic rates (Barnes 1995, Grange, Tyler et al. 2004). On this basis some authors have suggested that seasonality of metabolism should differ between trophic groups, with scavengers and predators showing less seasonal variation than primary consumers because their food supplies are more constant

through the year (Clarke 1988, Pearse, McClintock et al. 1991, Obermüller, Morley et al. 2010).

Several seasonal studies of metabolic rates in individual Antarctic species have been reported, including the holothurian *Heterocucumis steineni* (Fraser et al. 2004), the echinoid *Sterechinus neumayeri* (Brockington and Peck 2001) and the limpet *Nacella concinna* (Fraser et al. 2002). Few studies of seasonality of metabolic rate in Antarctic marine species involving more than a single species have been conducted, and these have been inconclusive as to whether predators and scavengers exhibit less seasonal variation (Obermüller, Morley et al. 2010). However, a recent study of metabolic seasonality in Antarctic demosponges (Morley, Berman et al. 2016) showed this group of primary consumers had the largest seasonal variation in metabolic rate so far reported.

The aim of this study was to document seasonal variation in oxygen consumption for five common benthic marine invertebrates present in Antarctic hard rock communities, over both austral summer and winter periods. We further aimed to test the hypothesis that seasonal changes in metabolic rates vary between species from different trophic levels, and that secondary consumers will exhibit less seasonal variation as they are less affected by seasonal variation in food supply than primary consumers. This study will also enable comparison of results from the same species; (*S. neumayeri*, *H. steineni*, *O. victoriae* and *N. concinna*) to those in previous seasonal metabolic studies in Antarctica over the last 18 years (Brockington and Peck 2001, Fraser, Clarke et al. 2002, Fraser, Peck et al. 2004, Obermüller, Morley et al. 2010). Long term time series are invaluable for identifying effects of climate change, and this is especially so in an area of rapid warming such as the Antarctic peninsula. Metabolic rate change over time would indicate responses to changing environments and the extra costs incurred. Long-term studies are further important in the Antarctic as they are one of the few ways of separating climate change impacts from natural cycles such as the Southern Ocean Oscillation or the El Niño and it can require decades of data before patterns become obvious.

This is the first study to examine the seasonal metabolic rates of the common Antarctic cushion star *O. validus*. This study will also allow physiological comparisons with environmental conditions, such as changes in temperature, duration of the summer phytoplankton bloom and duration of the winter fast ice. The metabolic rate data and therefore energy use, combined with diversity and biomass data and knowledge of trophic ecology of key species, could then be used to construct a preliminary energy flow diagram for the shallow rocky ecosystem around Rothera Point, Adelaide Island, Antarctica.

This study concentrates on the seasonality of metabolism of five common, locally abundant species. They are important components of the ecosystem and significant energy transformers (animals which transfer energy from one trophic level to another).

The species studied are:

1 The starfish *O. validus* (Figure 4.1) is found at an average density of 10.0 m⁻² around Cheshire Island, Rothera Point (Chapter 2). This asteroid is a common component of the benthic community. It is an opportunistic feeder with a varied diet, being reported to, graze on algae, suspension feed, scavenge (Pearse 1965) and actively predate other benthic invertebrates including cannibalism (Souster, unpublished obs). Its abundance and flexible feeding mode indicate that it is ecologically very important in the benthic ecosystem (McClintock, Pearse et al. 1988)



Figure 4.1 *O. validus* size scale 1:1, Photograph taken by Terri Souster

2 The echinoid *S. neumayeri* (Figure 4.2) is found at mean densities of 23m^{-2} (Chapter 2), but has been reported at densities up to 170m^{-2} nearby in South Cove, Rothera Point (Barnes and Brockington 2003). Its abundance makes it an important member of the Antarctic near shore community. *S. neumayeri* is an omnivorous, benthic pioneer species, occurring at high densities in recent iceberg scours, where a large portion of their diet comes from scavenging organisms (McClintock 1994).



Figure 4.2 *S. neumayeri* scale 1:2, Photograph taken by Terri Souster

3 The brittle star *O. victoriae* (Figure 4.3) is primarily a scavenger, but it is also an opportunistic generalist with a varied diet that can include cannibalism (Fratt and Dearborn 1984, McClintock 1994). It is one of the fastest moving benthic marine invertebrates in Antarctic shallow waters and it occurs at densities up to 5m⁻² (Chapter 2).

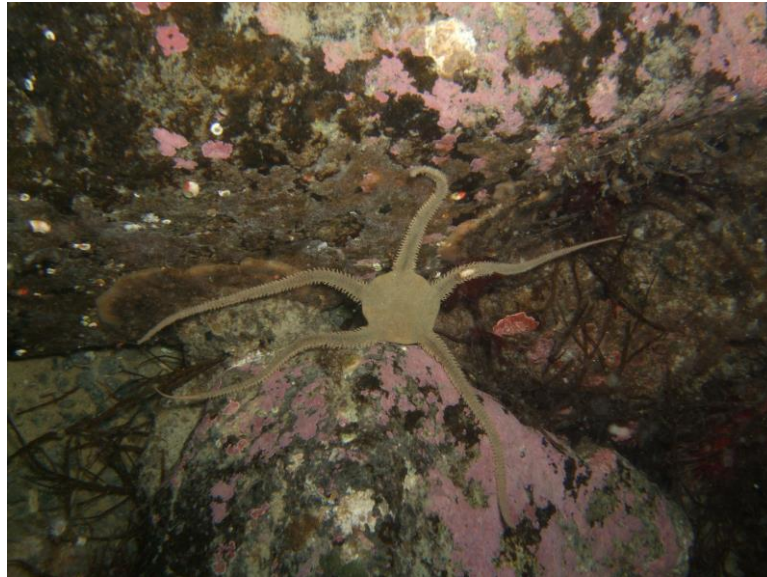


Figure 4.3 *O. victoriae* scale 1:2, Photograph taken by Terri Souster

4 The Antarctic limpet *N. concinna* (Figure 4.4) is abundant in both intertidal and subtidal waters to depths around 100m (Powell 1951, Walker 1972). *N. concinna* feeds all year round, and the diet consists mostly of diatoms and filamentous algae (Fraser et al. 2002), including endolithic algae growing in and on other limpet shells (Powell 1951, Nolan 1991).



Figure 4.4 *N. concinna* scale 1:1, Photograph taken by Terri Souster

5 The holothurian *H. steineni* (Figure 4.5) is one of the most common benthic marine invertebrates on shallow rocky habitats in Antarctica. In this study there were mean densities of 9m⁻² (Chapter 2). *H. steineni* is a suspension feeder which ceases feeding during the austral winter as it is dependent on suspended food particles, in particular diatoms which are only available during the summer phytoplankton blooms (Fraser 2004).



Figure 4.5 *H. steineni* scale 1:1, Photograph taken by Dr Gail Ashton

4.2 Methods

O. victoriae, *N. concinna*, *O. validus*, *H. steineni* and *S. neumayeri* were collected by hand with SCUBA during the austral summer (November – April) and the austral winter (June – October) from depths between 6m and 20m between Cheshire Island and South Cove at Rothera Point (Fig.1.2b Chapter 1). A wide size range of individuals of each species (Table 4.1) from juveniles to fully reproductive adults were collected to give a good representation of the routine metabolic rate across the population size range.

Species	WM range (g)	Number of individuals (n)	
		Summer	Winter
<i>O. victoriae</i>	1.87 – 7.69	23	33
<i>N. concinna</i>	0.51 – 8.65	27	29
<i>O. validus</i>	0.65 – 36.64	25	32
<i>H. steineni</i>	1.98 – 155.12	25	31
<i>S. neumayeri</i>	0.60 – 36.46	29	30

Table 4.1 Table of the wet mass (WM) range of individuals of the five species used to cover a size range of their population and the number of individuals of each species used to measure metabolic rate during both seasons.

After collection, individuals were returned to the laboratory where they were carefully sorted and any epibionts present removed. Great care was taken to ensure they remained submerged at all times. They were then held in an ambient flow-through aquarium for 48h, but for a maximum of 5 days, to allow for recovery from any collection and handling stress (but still allowing the measurement of routine metabolic rates as close to field values as possible).

Oxygen consumption measurements

For oxygen consumption measurements (MO_2), individual animals were placed in open chambers (volume dependant on species, size of individual and preliminary trials) with mesh lids (mesh 2mm – 5mm) and submerged in flow-through aquarium tanks for <8h prior to measuring oxygen consumption to allow individuals to adjust to experimental conditions. (Peck and Conway 2000 shows timecourse evaluation of oxygen consumption over the first 24 hours after animals were placed in respirometers, 6+ hours before oxygen consumption stabilises to routine). Thereafter the water inside the chamber was exchanged with seawater within the flow through tanks of the aquarium, and the chamber sealed ensuring no bubbles were present. Individuals of each species were placed in chambers scaled to animal size (using data from preliminary trials). For my preliminary trials specimens of different sizes were placed in different size chambers, and the rate of oxygen consumption (MO_2) was recorded. Chambers were chosen for a specific size of individual, which was appropriate to produce a reduction in oxygen concentration of approximately 15% of ambient over a 6 - 12 hour period depending on animal size and season. Experiments were conducted over an experimental period usually of 6-12 hours but not more than 20 hours depending on season, species, specimen size and volume of the respirometry chamber. Dissolved oxygen concentration in the chamber was recorded at the start and then every hour using a FIBOX-3 optode system. The species studied were all determined to be oxyregulators (*S. neumayeri*, from Brockington and Clarke (2001) and *O. victoriae*, *H. steineni*, *N. concinna* and *O. validus* from preliminary trials in this study (see Figures 4.6 - 4.9), i.e. their MO_2 is constant over a wide range of oxygen tensions. As each species is an oxyregulator closed chamber respirometry methods are appropriate for measuring oxygen consumption in these animals.

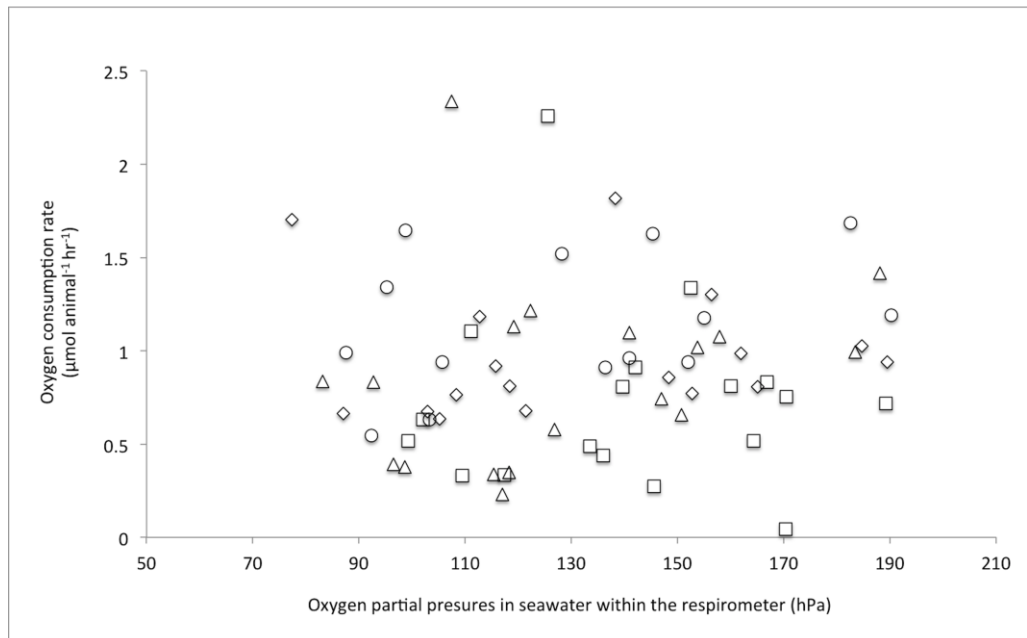


Figure 4.6. Oxyregulating behaviour of *O. victoriae* between 190 and 70hPa of oxygen partial pressure. The regression slope ($MO_2 = 0.6935 + 0.001620hPa$) was not significantly different from zero (ANOVA $F_{(1,67)} = 0.8$, $P = 0.374$). Four individuals (5.24g – 6.68g WM) are shown by different symbols.

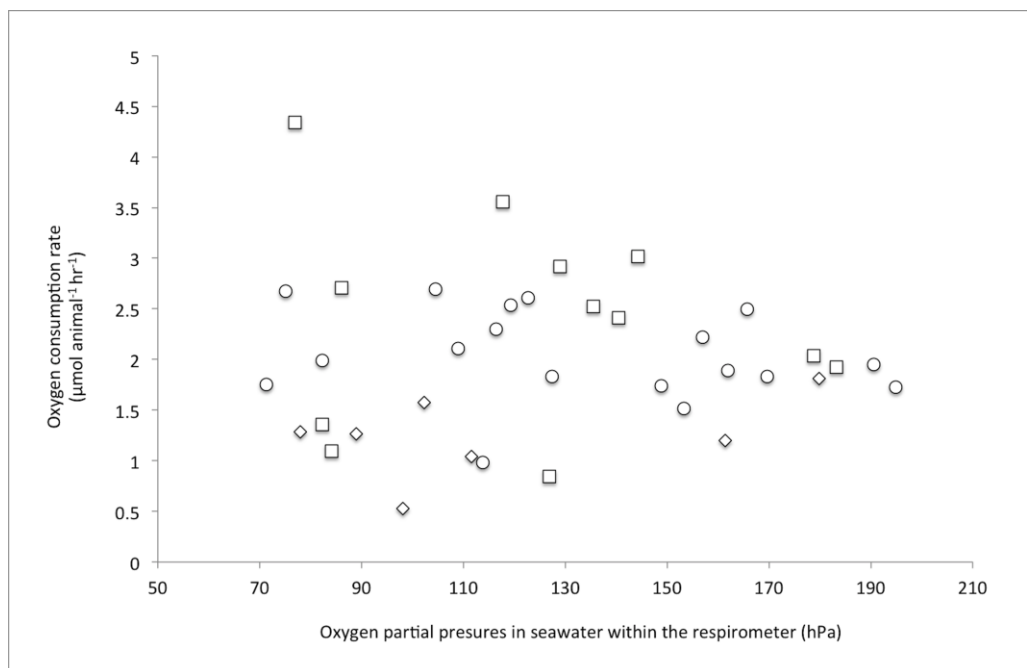


Figure 4.7. Oxyregulating behaviour of *O. validus* between 190 and 70hPa of oxygen partial pressure. The regression slope ($MO_2 = 2.093 + 0.00068hPa$) was not significantly different from zero (ANOVA $F_{(1,36)} = 0.04$, $P = 0.851$). Three individuals (18.75g – 38.98g WM) are shown by different symbols.

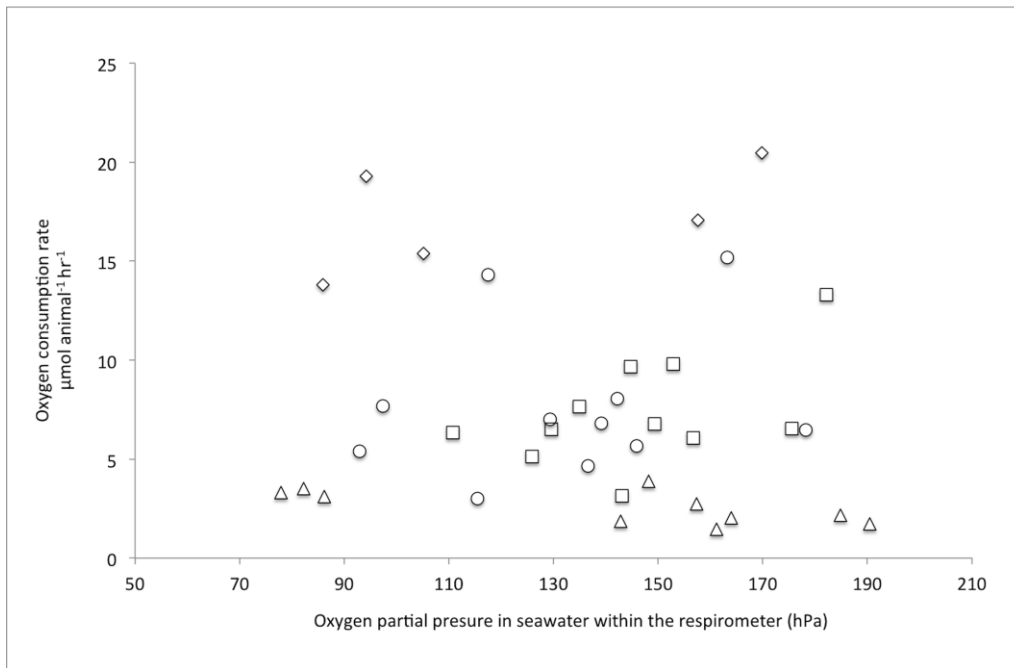


Figure 4.8. Oxyregulating behaviour of *H. steineni* between 200 and 70hPa of oxygen partial pressure. After square root transformation of oxygen data the regression slope ($MO_2 = 2.919 - 0.00244hPa$) was not significantly different from zero (ANOVA $F_{(1,36)} = 0.3$, $P = 0.622$). Four individuals (3.13g – 8.78g WM size range) are represented by different symbols.

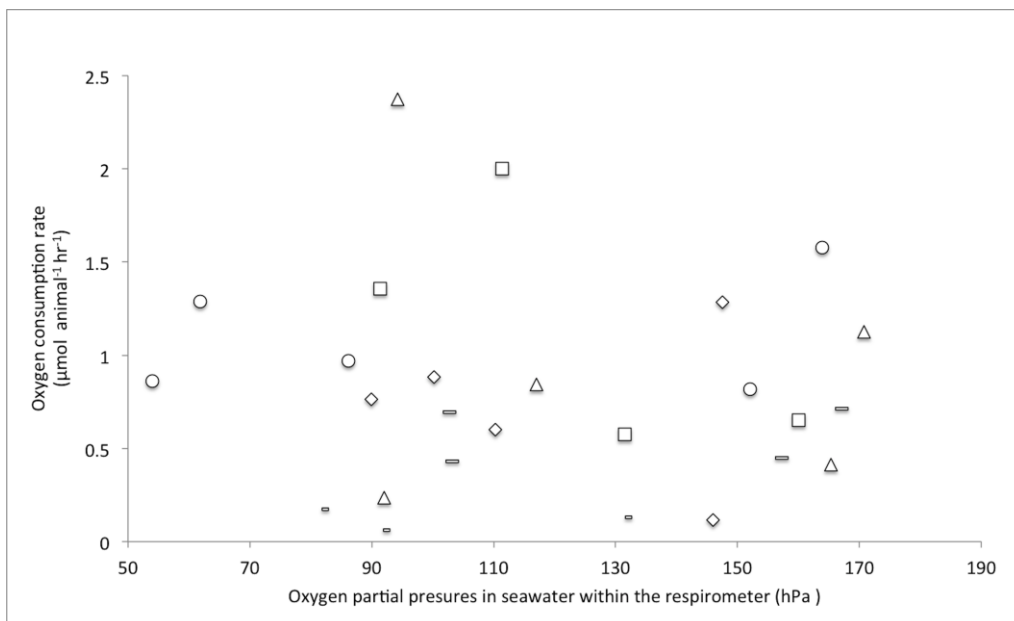


Figure 4.9 Oxyregulating behaviour of *N. concinna* between 200 and 50hPa of oxygen partial pressure. The regression slope ($MO_2 = 0.975 - 0.00129hPa$) was not significantly different from zero (ANOVA $F_{(1,25)} = 0.2$, $P = 0.705$). Six individuals (0.75g – 10.2g) are shown by different symbols.

In all experiments, respirometer volume was corrected for the volume of water displaced by the animal and oxygen consumption [$\mu\text{mol O}_2 \text{ g ash free dry mass (AFDM)}^{-1} \text{ h}^{-1}$] was adjusted by comparison with control chambers (without animals). Whole-animal dry mass (DM) was measured after drying to a constant mass at 60°C and ash mass (AM) was obtained after incineration in a muffle furnace at 475 °C for 12 h (juveniles) and 24 h (adults.) AM was subtracted from DM to obtain AFDM for each specimen.

There are a number of ways of comparing metabolic rate data: One is to compare the slope and elevation of the line fitted to the relationship between oxygen consumption and size, and another is to compare the metabolic rate for an animal of standard mass. Both of these methods allow comparison with other metabolic data and they minimise the influence of outliers. For this study, a standard animal mass of 0.3g AFDM was used for comparisons as this mass was within the range of all five species studied.

Statistics

Analysis in this chapter compared two or more sets of continuous heterogeneous data and investigated the effect of two factors; season and size (AFDM). Therefore the appropriate statistical test under parametric conditions was a general linear model (GLM). Observations were independent, both within and between samples. Prior to the GLM analysis, data were tested for normality using the Anderson-Darling test. Non-normal data were transformed logarithmically to achieve normality then the normalized data were analysed using the GLM package in MINITAB version 17 for Windows. As larger individuals have a higher metabolic rate, all GLMs included size as a covariate, however as it is well known that larger animals have a higher metabolic rate than smaller animals, the effect is not described within the results section. Comparison of oxygen consumption between species were based on a standard animal mass (0.3g AFDM) using a one way ANOVA to determine difference between the means of the different species. If the ANOVA showed a significant difference, a *post hoc* Fisher's pairwise comparison was conducted to

determine which species were different. Statistical results from the post hoc Fisher's pairwise were displayed with the lowest T value. To identify differences in metabolic rate between seasons in the same species a Two Sample T Test was used.

4.3 Results

Across all five species, 124 measurements of oxygen consumption were made during the austral summer and 150 during the austral winter. There was a significant difference between seasons for *S. neumayeri* and *O. validus* (Figures 4.10, 4.11) consistent across the size range in this study but no similar consistent metabolic seasonality in *N. concinna*, *O. victoriae* or *H. steineri* (Figures 4.12, 4.13, 4.14 respectively) across sizes. The relationship between metabolic rate and mass (slope) was significantly different between summer and winter for *N. concinna* and *H. steineri* indicating there was no consistent significant seasonal difference across the size range (Figures 4.12 and 4.14).

Sterechinus neumayeri

Comparing seasonality for *S. neumayeri*, the slope of the relationship between Ln metabolic rate and urchin size (Ln AFDM) was not significantly different from summer to winter (GLM $F_{(1,58)} = 0.01$, $P = 0.930$). *S. neumayeri* had a significant seasonal difference in oxygen consumption between summer and winter across the whole size range studied (GLM, $F_{(1,58)} = 6.12$, $P = 0.017$ Table 4.2). The oxygen consumption in *S. neumayeri* in summer was 1.39 times higher or 39% higher (summer intercept 1.32 – winter intercept 1.00 = 0.32 which when antilogged is 1.39) than the winter. The metabolic scaling coefficient for *S. neumayeri* in this study was $0.89 \pm \text{SE } 0.06$, which was significantly different to 0.75 and 1.0 (based on non overlap of 95% confidence intervals). Comparing a standard animal of 0.3g AFDM had the largest numerical decrease in metabolic rate from summer to winter compared with the other four species from $1.57 \mu\text{mol O}_2 \text{ h}^{-1} \pm \text{SE } 0.15$ to $1.08 \mu\text{mol O}_2 \text{ h}^{-1} \pm \text{SE } 0.06$ (Figure 4.15).

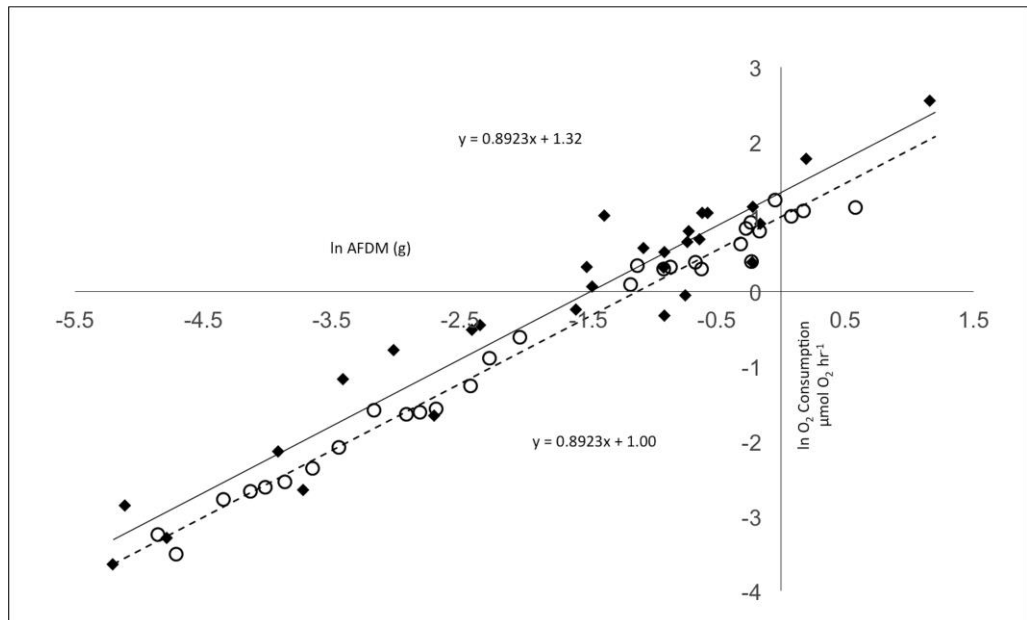


Figure 4.10. Seasonal metabolic rates for the Antarctic urchin *S. neumayeri*. Oxygen consumption and size (AFDM) are presented for the austral summer (Jan-March 2015 N = 29 $\mu\text{mol O}_2 \text{ h}^{-1}$ \blacklozenge) and austral winter (June - October 2015 N= 30 $\mu\text{mol O}_2 \text{ h}^{-1}$ \circ) solid line = summer regression, dotted line = winter regression.

Odontaster validus

For the cushion star *O. validus* the slopes of the oxygen consumption relationships with AFDM were not significantly different between summer and winter (Figure 4.11) (GLM $F_{(1,56)} = 1.99$, $P = 0.16$). There was however, a significant seasonal difference in oxygen consumption between summer and winter across the size range studied (GLM $F_{(1,56)} = 15.03$, $P < 0.001$, Table 4.2). The oxygen consumption in *O. validus* in summer was 1.44 times or 44% higher (summer intercept 0.95 – winter intercept 0.59 = 0.36 which when antilogged is 1.44) than the winter. The metabolic scaling coefficient for *O. validus* in this study was 0.89 (\pm SE 0.06), which was significantly different to 0.75 and 1.0 (based on non overlap of 95% confidence intervals). Using a different comparison method of standard animal mass, *O. validus* of 0.3g AFDM showed a decrease in metabolic rates from summer to winter of $0.93 \mu\text{mol O}_2 \text{ h}^{-1}$ (\pm SE 0.15) to $0.63 \mu\text{mol O}_2 \text{ h}^{-1}$ (\pm SE 0.024) (Figure 4.15).

In the cushion star *O. validus*, there was a significant positive relationship between oxygen consumption and AFDM in both summer and winter (GLM $F_{(1,56)} = 1458.58$, $P < 0.001$). There was also a significant seasonal difference in oxygen consumption (GLM $F_{(1,56)} = 15.03$, $P < 0.001$). However the slopes of the relationship in summer and winter were not significantly different (GLM $F_{(1,56)} = 1.99$, $P = 0.16$). The intercept, based on a common slope, for the summer data is 0.95 and for the winter data is 0.59; therefore, for all sizes of *O. validus*, in this study, the metabolic rates were $(0.954 - 0.5916) 0.36 \mu\text{mol O}_2 \text{ h}^{-1}$ higher in summer than in winter (Figure 4.12), compared to the $0.30 \mu\text{mol O}_2 \text{ h}^{-1}$ obtained using the standard animal approach.

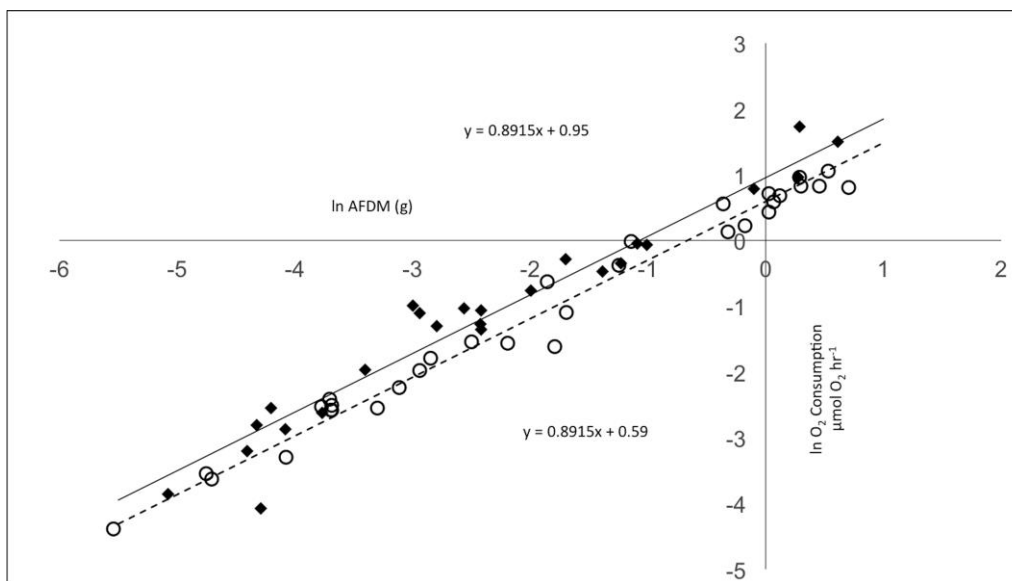


Figure 4.11 Seasonal metabolic rates for the Antarctic cushion star *O. validus*. Oxygen consumption and size (AFDM) are presented for the austral summer (Jan-March 2015 N = 25 ◆) and austral winter (June – October 2015 N= 32 ○) solid line = summer regression, dotted line = winter regression.

Nacella concinna

In the limpet *N. concinna*, the slope of the relationship between Ln metabolic rate and size (AFDM) is significantly different in summer compared with winter (GLM $F_{(1,55)} = 5.50$, $P = 0.023$ Table 4.2). The seasonal effect on oxygen consumption appears different in large *N. concinna* compared to small *N. concinna* (Figure 4.12). However when comparing seasonality in the small animals using GLM analysis, there was no significant difference (GLM $F_{(1,18)} = 2.50$, $P = 0.131$). For seasonal comparison using a standard animal of *N. concinna* of 0.3g AFDM, there was no seasonal difference (Figure 4.15) and the rate of oxygen consumption was $1.3 \mu\text{mol O}_2 \text{ h}^{-1}$ in both seasons.

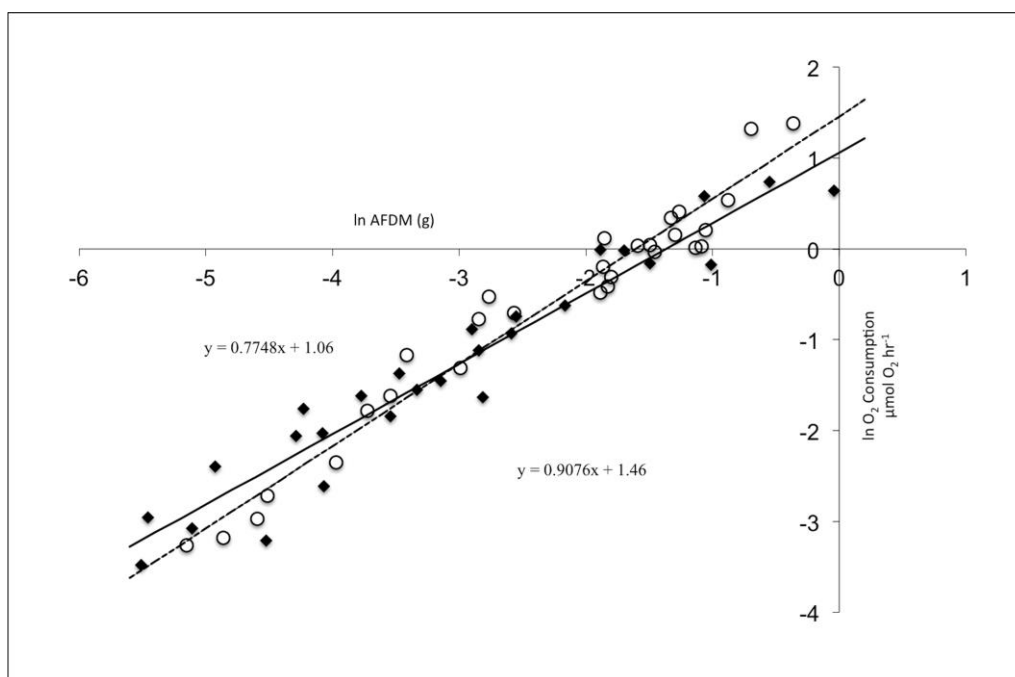


Figure 4.12 Seasonal metabolic rates of the Antarctic limpet *N. concinna*. Oxygen consumption and size (AFDM) are presented for the (Jan-March 2015 $N = 27$ ◆) and austral winter (June – October 2015 $N = 29$ ○), solid line = summer regression, dotted line = winter regression.

Ophionotus victoriae

Comparing seasonality in the brittle star *O. victoriae*, the slope of the relationship between \ln metabolic rate and size (\ln AFDM) was not significantly different in summer compared with winter (GLM $F_{(1,55)} = 0.37$, $P = 0.55$, Table 4.2) and there was also no significant seasonal difference in the intercepts of the relationships (GLM $F_{(1,55)} = 2.14$, $P = 0.15$), therefore a single regression line was used (Figure 4.13). Furthermore, the residuals for each season in the combined regression were not significantly different from each other (T test $P = 0.367$, $n = 56$).

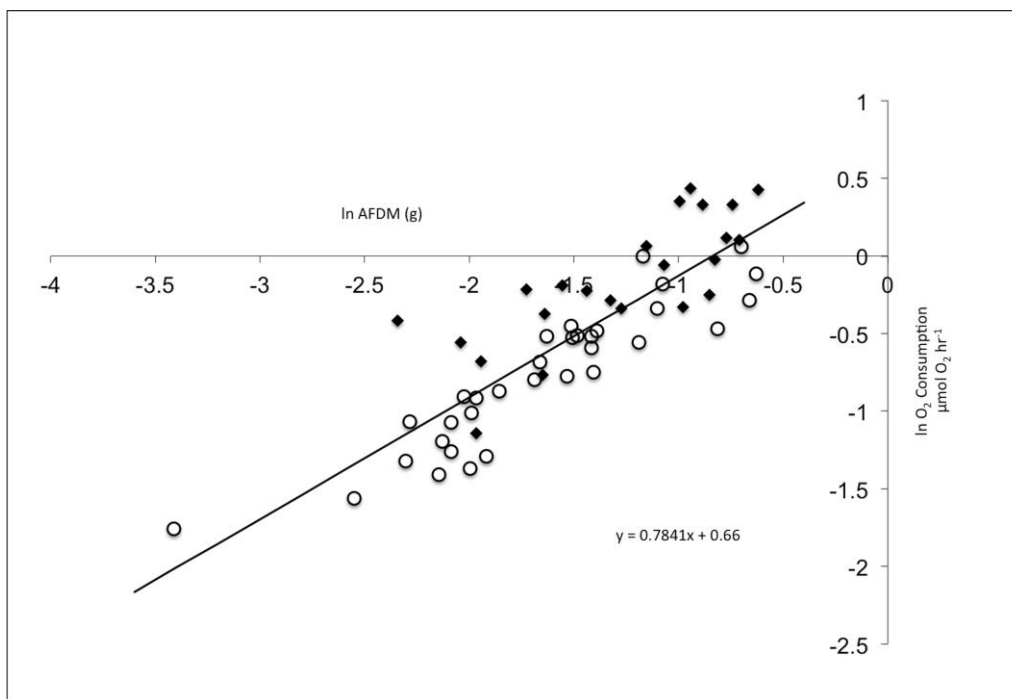


Figure 4.13 Seasonal metabolic rates of the Antarctic brittle star *O. victoriae*. Oxygen consumption and size (AFDM) are presented for the austral summer (Jan-March 2015 N = 23 ◆) and austral winter (June – October 2015 N= 33 ○).

Heterocucumis steineni

Comparing seasonality in the holothurian *H. steineni* the slope of the relationship between Ln metabolic rate and size (AFDM) is significantly different in summer compared with winter (GLM $F_{(1,55)} = 7.00$, $P = 0.011$). Seasonal differences across the size range studied could not therefore, be assessed by comparing regression lines, but it should be noted that the summer and winter regression lines cross in the juvenile size range (Figure 4.14). When testing metabolic rate for a standard animal, mass corrected to 0.3g AFDM however, there is a significant seasonal difference (T test $T = 6.39$, $P < 0.001$ Figure 4.15). This indicates that seasonal differences exist for large, but not small individuals.

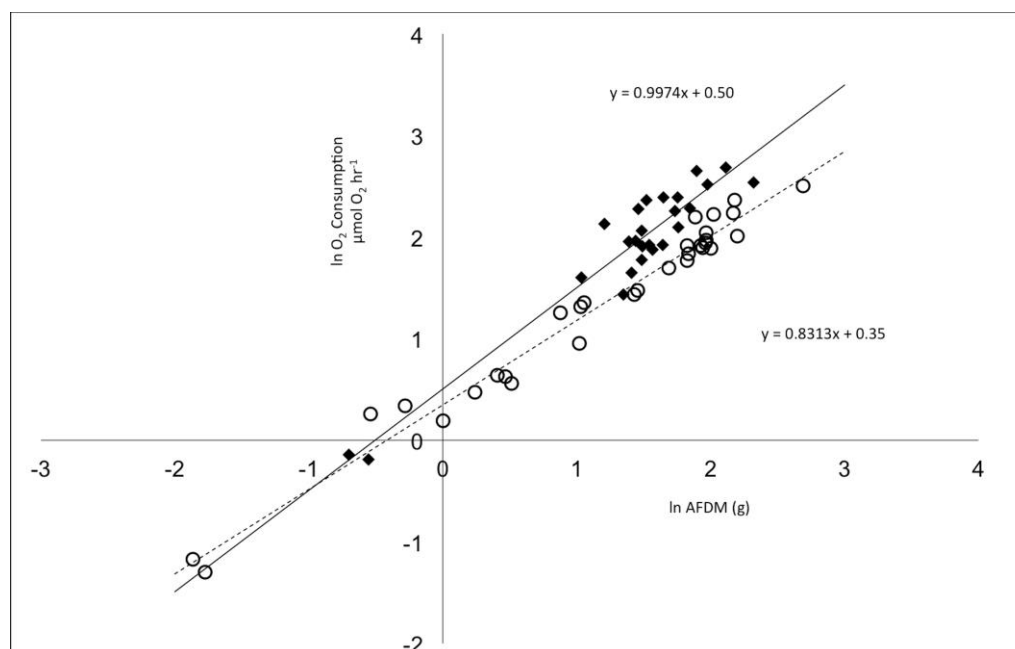


Figure 4.14 Seasonal metabolic rates of the Antarctic holothurian *H. steineni*. Oxygen consumption and size (AFDM) are presented for the austral summer (Jan-March 2015 N = 25 ◆) and austral winter (June – October 2015 N= 31 ○), solid line = summer regression, dotted line = winter regression.

Species	Covariate	Intercept	Slope
<i>S. neumayeri</i>	$F_{(1,58)} = 1151.93$, $P < 0.001$	$F_{(1,58)} = 6.12$, $P = 0.017$	$F_{(1,58)} = 0.01$, $P = 0.930$
<i>O. validus</i>	$F_{(1,56)} = 1458.58$, $P < 0.001$	$F_{(1,56)} = 15.03$, $P < 0.001$	$F_{(1,56)} = 1.99$, $P = 0.16$
<i>N. concinna</i>	$F_{(1,55)} = 882.18$, $P < 0.001$	$F_{(1,55)} = 5.33$ $P = 0.025$	$F_{(1,55)} = 5.50$, $P = 0.023$
<i>O. victoriae</i>	$F_{(1,55)} = 136.50$, $P < 0.001$	$F_{(1,55)} = 2.14$, $P = 0.15$	$F_{(1,55)} = 0.37$, $P = 0.55$
<i>H. steineni</i>	$F_{(1,55)} = 847.42$, $P < 0.001$	$F_{(1,55)} = 2.47$, $P = 0.122$	$F_{(1,55)} = 7.00$, $P = 0.011$

Table 4.2 Results of GLM, two factors: season and size (AFDM), Slope – rate of metabolic change with respect to mass (AFDM), Intercept – Effect of season on metabolic rates, Covariate – effect of mass (AFDM) on metabolic rate

Comparisons between species

When the slope is not significantly different between seasons, the intercept can be used to estimate seasonal differences in metabolic rates as with *S. neumayeri* and *O. validus*. However, this approach was not applicable in the other species because the slopes of winter and summer regressions were significantly different and a standard animal approach was thus used to assess differences between species. When comparing seasonal oxygen consumption between the five species for a standard animal of AFDM 0.3g then all the species studied showed significant seasonal differences; *O. victoriae* (T test, $T = 4.68$, $P < 0.001$), *H. steineni* (T test, $T = 6.39$, $P < 0.001$), *O. validus* (T test, $T = 4.38$, $P < 0.001$) and *S. neumayeri* (T test, $T = 3.04$, $P < 0.05$) except *N. concinna* which showed no significant seasonal difference (T test, $T = -0.36$, $P = 0.718$ Figure 4.15).

Comparisons of rates of oxygen consumption for a standard animal between species in summer showed there were significant differences ANOVA ($F_{(1,128)} = 16.05$, $P < 0.001$). A post hoc Fisher's pairwise comparison test showed for a standard animal of 0.3g AFDM, the metabolic rates of *H. steineni* were significantly different to *N. concinna*, *S. neumayeri*, *O. victoriae* and *O. validus* (post hoc $T > 1.99$, $P < 0.05$). *O. validus* was significantly different to *S. neumayeri* and *N. concinna* (post hoc $T > 3.22$, $P = 0.002$). *S. neumayeri* and *N. concinna* were significantly different to *O. victoriae* (Post hoc $T = -4.86$, $P < 0.001$, Post hoc $T = -3.12$, $P < 0.001$ respectively) Figure 4.15. *S. neumayeri* also had the highest MO_2 in the summer ($1.57 \mu\text{mol O}_2 \text{ h}^{-1}$), followed by *N. concinna* ($1.35 \mu\text{mol O}_2 \text{ h}^{-1}$) then both *O. victoriae* ($0.93 \mu\text{mol O}_2 \text{ hr}^{-1}$) and *O. validus* ($0.93 \mu\text{mol O}_2 \text{ h}^{-1}$), which were not significantly different and *H. steineni* had the lowest rate ($0.67 \mu\text{mol O}_2 \text{ h}^{-1}$).

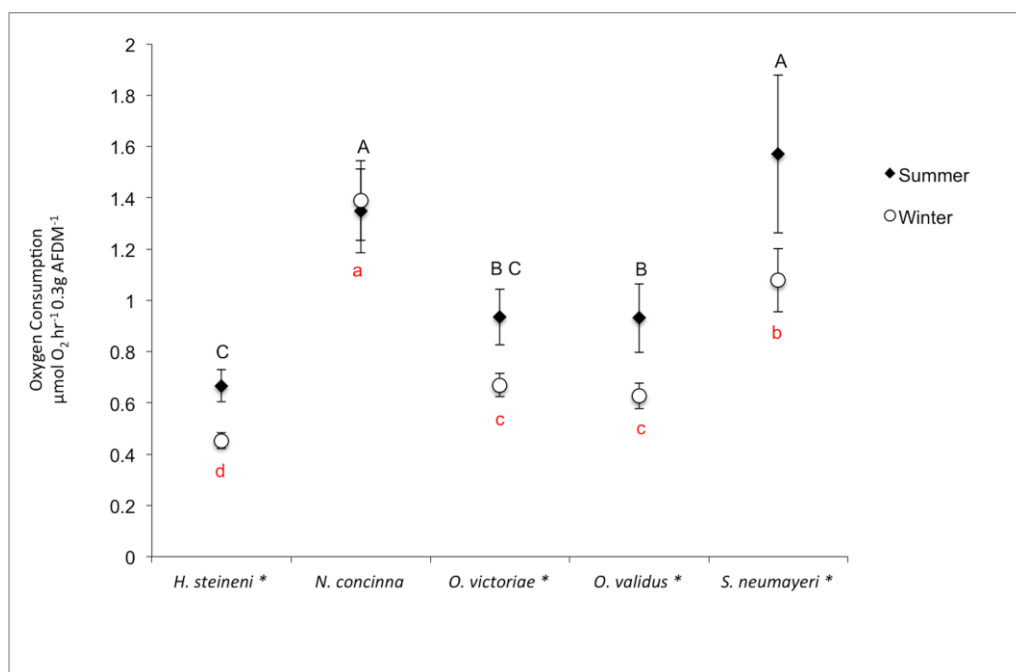


Figure 4.15 Mean metabolic rate for a standard animal of AFDM 0.3g for five Antarctic benthic marine invertebrates for summer (Jan – March 2015) and winter (June – October 2015) \pm 95% CI. Species with the same capital letter above have no significant difference in summer metabolic rates. Species with the same small red letter below have no significant difference in their winter metabolic rates. * indicates a significant seasonal difference within species.

Comparisons of rates of oxygen consumption for a standard animal between species in winter showed there were significant differences (ANOVA $F_{(1,54)} = 71.63$, $P < 0.001$). A post hoc Fisher's pairwise comparison test showed the metabolic rates of *H. steineni* were significantly different to *N. concinna*, *S. neumayeri*, *O. victoriae* and *O. validus* (all Post hoc $T > 2.81$, $P < 0.05$). *O. validus* and *O. victoriae* were significantly different to *S. neumayeri* and *N. concinna* (all Post hoc $T > 7.81$, $P < 0.001$). *S. neumayeri* was significantly different to *N. concinna* (Post hoc $T > 4.83$, $P < 0.001$). *N. concinna* had the highest MO_2 in winter ($1.39 \mu\text{mol O}_2 \text{ h}^{-1}$), followed by *S. neumayeri* ($1.08 \mu\text{mol O}_2 \text{ h}^{-1}$), then *O. victoriae* ($0.67 \mu\text{mol O}_2 \text{ h}^{-1}$) and *O. validus* ($0.63 \mu\text{mol O}_2 \text{ h}^{-1}$), which were not significantly different, and *H. steineni* had the lowest rate ($0.45 \mu\text{mol O}_2 \text{ h}^{-1}$).

4.4 Discussion

Antarctic shallow water habitats are characterised by extreme seasonal environmental variation in light, ice and phytoplankton productivity. The data from this study are within the previously observed range for seasonal factorial changes in oxygen consumption of polar marine species (Figure 4.16).

Of the primary consumers previously studied there was a range of seasonal factorial rises in metabolic rate between 0.8 for the Porifera *Clathra nidificata* and 5.5 for *Suberites* sp, which demonstrates the diversity possible within just one trophic level and one taxonomic group. It was predicted that *H. steineni* (the primary consumer in this study) would have the largest factorial change due to the highly seasonal food supply in the austral phytoplankton bloom compared with secondary consumers, some of which can continue to feed during winter. This was however, not the case as the factorial change for *H. steineni* was similar to *S. neumayeri* and *O. validus* (both are opportunistic scavengers), despite the fact that *H. steineni* stops feeding for 4-6 months in a typical winter (Fraser et al. 2004). The factorial change for *H. steineni* from this study (1.5) was similar to that reported by Fraser et al. (2004) of 2.0. It is therefore unlikely this was an unusual year for the primary consumer.

However, there is flexibility in seasonality of feeding as the grazer/scavenger urchin *S. neumayeri* ceases feeding for 4-7 months of the year (Brockington, Clarke et al. 2001), and not all primary consumers cease feeding during the winter. For example the bryozoan *Arachnopusia inchoata* feeds all year round (Barnes and Clarke 1995), but the Antarctic clam *Laternula elliptica* has low energy requirements during the winter and so ceases to feed (Morley, Peck et al. 2007). Although food availability could be the main driver in the effect of season on metabolic rate within species, there are other variables that could explain the diversity of results within the different trophic levels.

Physical factors affecting the physiology of Antarctic benthic marine invertebrates include photoperiod, temperature (Clarke and Brockington 2001), sea ice cover (Conover and Huntley 1991, Arrigo, van Dijken et al. 2008), salinity (Gyllenberg and Lundqvist 1979), acidification (Seibel, Oschlies et al. 2012) and sedimentation from nearby glaciers (Torre, Servetto et al. 2012). Biological factors affecting physiological mechanisms are phytoplankton bloom (Peck, Brockington et al. 1997), predation pressure (Seibel and Drazen 2007), competition (Seibel and Drazen 2007), activity (Whitney, Lear et al. 2016), feeding (Chapelle, Peck et al. 1994), reproduction (Grange, Tyler et al. 2004, 2007) etc. Antarctic seasonal factors such as sea temperature vary by $<0.2^{\circ}\text{C}$ in the highest latitude sites. e.g. McMurdo Sound (Clarke 1988) and as little as 3°C at Rothera. In this study, *S. neumayeri*, *H. steineni*, *N. concinna* and *O. victoriae*, had oxygen consumption rates similar to previous reported values (Brockington and Peck 2001, Fraser, Clarke et al. 2002, Fraser, Peck et al. 2004, Obermüller, Morley et al. 2010, 2011) and are low compared to species from temperate and tropical localities.

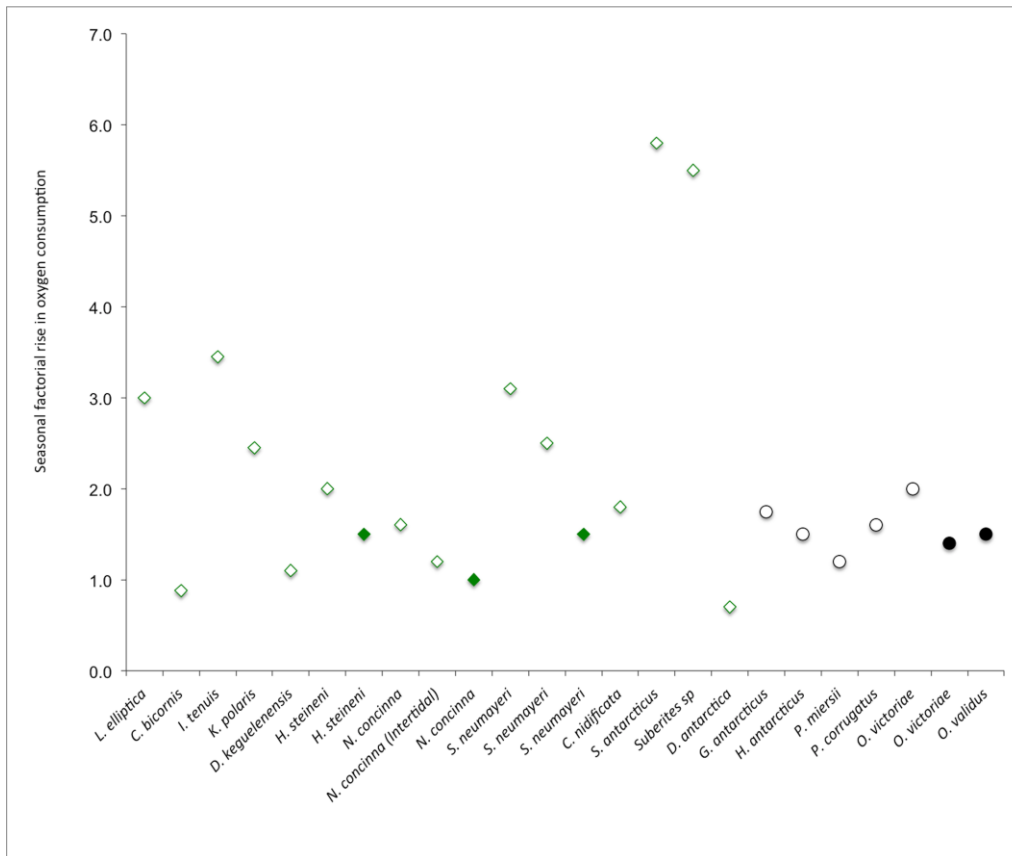


Figure 4.16 Comparison of seasonal factorial rise in oxygen consumption from winter to summer in a range of polar marine species. Modified from Barnes & Peck (2005), Obermüller et al. (2010) and Morley et al. (2016). Open symbols represent data from previous studies, ◆ = herbivores • = scavengers/carnivores: Sources for data: *Laternula elliptica* (Peck and Brockington 2001), *Camptoplites. bicornis*, *Isosecuriflustra tenuis* and *Kymella polaris* (Barnes & Peck 2005), *Doris kerguelensis* (Obermüller et al. 2010), *Heterocucumis steineni* (Fraser et al. 2004) *Nacella concinna* (Fraser et al. 2002), *Nacella concinna* (intertidal) (Obermüller et al. 2010), *Stereochonis neumayeri* (Peck and Brockington 2001), *Glyptonotus antarcticus* (Janecki 2006), *Harpagifer antarcticus*, *Paraceradocus miersii*, *Parbolasia corrugatus* and *Ophionotus victoriae* (Obermüller et al. 2010). Closed symbols are data from the present study (*Heterocucumis steineni*, *Nacella concinna*, *Stereochinus neumayeri*, *Ophionotus victoriae* and *Odontaster validus*).

The metabolic cold adaptation (MCA) hypothesis, states that ectotherms living at low temperatures should show elevated metabolic rates to overcome the problems of performing activities at low temperatures (Milleikovsky 1971). This has not been substantiated by this study. If there was MCA then the polar animals should show higher metabolic rates, or at least rates similar to temperate species, which is not the case. This finding agrees with several previous studies. Fraser et al. (2004) showed rates of oxygen consumption in a range of holothurian species from tropical, temperate and polar habitats and *H. steineni* had the lowest metabolic rate of all the holothurians investigated. The rate reported here for *H. steineni* is similar to that reported by Fraser et al. 2004, and hence a similar conclusion can be drawn.

Furthermore, Peck (2016), in a comparison of oxygen consumption in bivalve molluscs from the tropics to the poles, showed rates in polar species matched those predicted from lower latitude species using an Arrhenius relationship. Hence there was no apparent MCA. Other studies comparing metabolic rates in Antarctic marine animals with those from lower latitudes have also found no evidence of MCA including; Luxmoore (1984) for isopods, Ralph and Maxwell (1977) for the bivalve *Gaimardia*, Clarke and Johnston (1999) for perciform fish and Peck and Conway (2000) for bivalve molluscs. There are also however, several studies that support the MCA concept (e.g. Wohlschlag 1964, Rastrick and Whiteley 2013). Some studies reporting MCA have been criticised on methodological grounds (Rakusa-Suszczewski 1982), but some studies show MCA for biochemical and mitochondrial processes, e.g. White, Alton et al. (2011) and within species latitudinal studies often seem to show MCA.

As Obermüller et al. (2010) found there is a remarkable degree of diversity in physiological strategies, cold water adaptations and degree of seasonality within Antarctic marine invertebrates. Further insight can be gained from a species by species analysis to examine the underlying patterns:

Sterechinus neumayeri

S. neumayeri had the largest seasonal decrease in metabolic rates compared to *N. concinna*, *O. validus*, *H. steineni* and *O. victoriae* for the size range of animals used in this study (Figure 4.15). The Antarctic urchin is a generalist omnivore that feeds mainly on diatoms and algae that are seasonal. It also however, consumes foraminiferans, amphipods, bryozoans and sponges, which are present all year round (Brockington 2001). Brockington (2001) showed that *S. neumayeri* ceased feeding for 7 months during the winter of 1997 and 4 months during the winter of 1998, which was accompanied by loss of locomotor activity.

There is a cost to feeding and therefore *S. neumayeri* may choose not to feed at all even though food is present. The benefit of feeding would depend on both the quality and quantity of available food. The quality and quantity of food arriving at the seabed in the Antarctic has several effects on the composition and condition of tissues in marine invertebrates (Grange 2005). Comparing the seasonal data from this project to seasonal metabolic data collected by Brockington (2001) from 1999 for a standard animal of 0.88g AFDM (Figure 4.16), shows there is large interannual variation in metabolic rates for *S. neumayeri*, but both years showed similar seasonal change between summer and winter. The summer of 1999 had over double the amount of chlorophyll a recorded in the water column (RATS long term data series) compared with the summer of 2015 (Figure 4.18). This would mean the summer of 1999 had a stronger phytoplankton bloom, which would restrict the amount of light penetration through the water column and therefore affect the amount of benthic productivity. As *S. neumayeri* feeds on both benthic diatoms and depositing plankton, both the quality and quantity of the food available

could be a reason for the higher metabolic rates recorded in the summer of 2015 compared with summer of 1999.

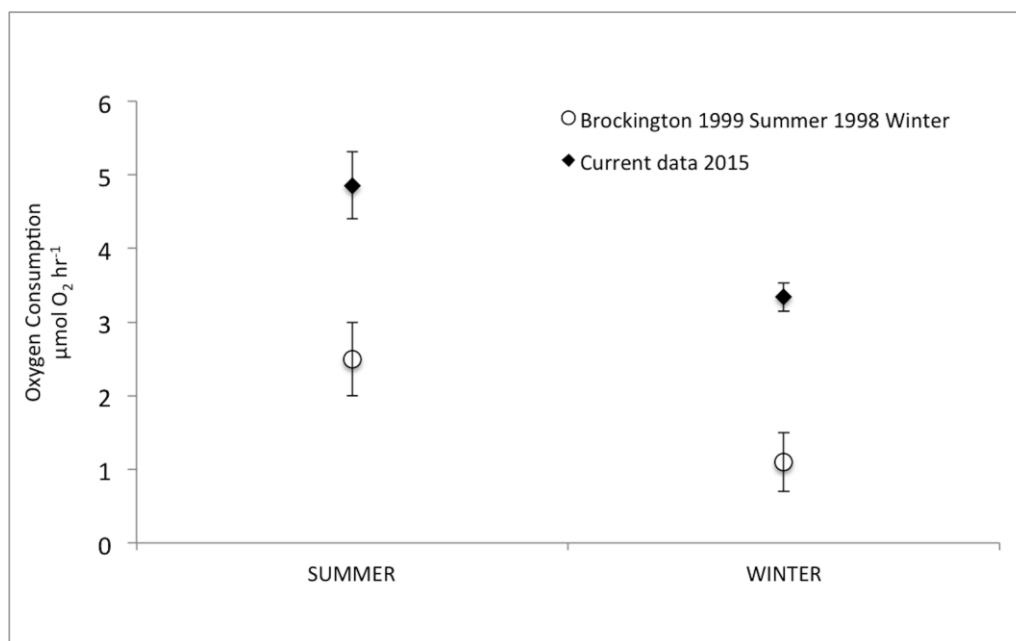


Figure 4.17 Oxygen consumption in *S. neumayeri* between seasons and years for a mass corrected individual (0.88g AFDM). Data from Brockington (2001) and this study.

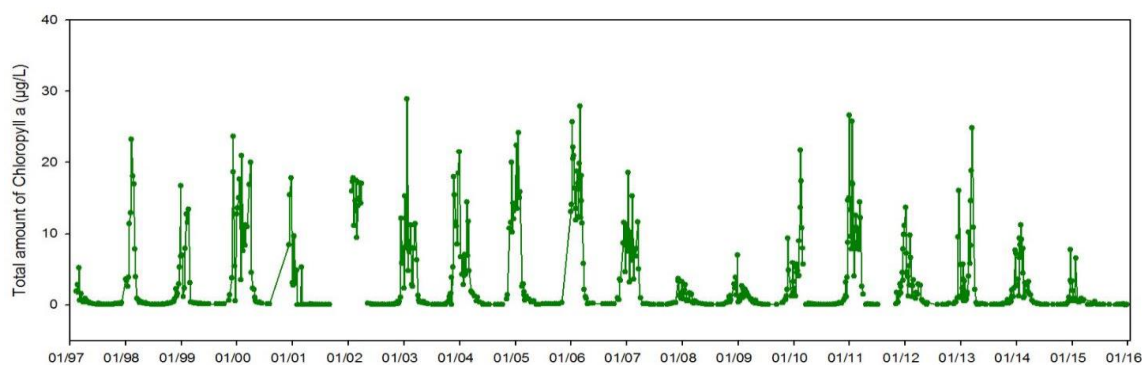


Figure 4.18 Annual changes in the chlorophyll a concentration (mg l^{-1}) at 15m depth in Ryder Bay from 1997 to 2015 (RATS unpublished data Hugh Venables).

Brockington et al. (2001) made his metabolic measurements in winter in October 1998 and made summer measurements in January 1999, whereas summer measurements in this study were carried out between January – March 2015 and winter samples covered a longer period from June to October 2015. Brockington et al. (2001) showed a significant seasonal difference in metabolic rates for *S. neumayeri* as found here, but the seasonal factorial rise in oxygen consumption from winter to summer was a lot higher, 3.1 in 1997 and 2.5 in 1998 compared with this study which was 1.5 (Figure 4.16). This could be explained by the size range of the urchins used in Brockington's work that were between 24.9mm and 39.6mm which means all would have been large reproductive adults.

Spawning occurs in *S. neumayeri* in October to January and is strongest in November (Brockington et al. 2001), and the timing of spawning ensures that feeding larvae are in the plankton during the summer phytoplankton bloom as observed at this site by (Bowden, Clarke et al. 2009). Brey (1995) found that more than 95% of production in adult *S. neumayeri* was invested in reproduction and less than 5% in somatic growth. Growth bands on the Aristotle's lantern are not formed annually and *S. neumayeri* grows slowly reaching its maximum diameter at 40 years of 70mm. A significant portion of the interannual differences in metabolic rates could be due to timing of the study; whether the animals were pre or post spawning, or whether they were in active gametogenesis, whether they were feeding and if they were growing. A small change in timing could have a large effect on the measured seasonal change in metabolic rate.

In this study the size range of urchins used was between 5.84mm and 45.48mm, which includes juveniles and so the factorial rise from winter to summer might be less pronounced. The techniques used in the two studies to measure oxygen consumption are also different. Brockington et al. (2001) measured urchin metabolic rate using a fuel cell based technique described by Peck and Uglow (1990), whereas measurements in this study were made using a FIBOX 3 OPTODE system. Both studies assessed routine metabolic rates and the experimental protocol was the same.

Differing methods could therefore help to explain some of the higher metabolic values for summer and winter compared with Brockington et al. (2001), but other factors are more likely. Could this difference be due to climate change and ocean warming since 1998? The sea on the western Antarctic Peninsula has warmed by 1°C (Meredith and King 2005), in the second half of the 20th century. This temperature increase was also accompanied by an increase in iceberg scour (Barnes and Souster 2011) and a decrease in macroalgal abundance. There is however, a need for further data to be able to reliably identify any effect of environmental change.

Reproductive cycles can have multi- year periodicities (Grange, Tyler et al. 2007). Some Antarctic marine invertebrates also have individual specific multi annual growth cycles, thought to be linked to reproductive cycles. Therefore in some years in summer, some adults will spawn and others might not, which would add variability to any metabolic rates measured on adult urchins. We know from studies of reproduction in other Antarctic echinoderms such as the brittle star *O. victoriae* that gonad index varies markedly between years with some years being particularly poor reproductive years (Grange, Tyler et al. 2007). Poor reproductive years could coincide with low food supplies, poor quality food or even high competition for food. In 1998, a biodiversity survey of the same areas studied in this thesis showed a much higher population density of *S. neumayeri* (Barnes and Brockington 2003) compared with 2015 summer (Chapter 3).

In 1998, at 6m there were 148 urchins m⁻² compared with 16.8 in 2015, at 12m there were 493.3 urchins m⁻² compared with 23.2 in 2015 and at 20m there were 498 urchins m⁻² compared with 28.8. These figures show there were roughly 10 -20 times as many urchins inhabiting rocky seabed sites in this area 15-20 years ago. There would therefore have been more competition for food and space in 1998. The research reported here contained urchins from a wide size range to be representative of the majority of the shallow water urchin population.

The smaller urchins in the lower density population compared with 1998, would possibly have had greater access to food as there would have been less intraspecific competition which could explain the higher metabolic rate compared with Brockington (2001) (Figure 4.16). Bowden (2005) also found that while large urchins may cease feeding during winter, small individuals by contrast are able to exploit sessile faunal assemblages in cryptic habitats between and under rocks but this study (Figure 4.10) shows the same metabolic seasonality for adults and juveniles.

Odontaster validus

High Antarctic shallow waters are often dominated by the Echinodermata, especially the echinoid *S. neumayeri*, the ophiuroid *O. victoriae* and the seastar *O. validus*. (McClintock, Pearse et al. 1988, Brockington, Clarke et al. 2001, Moya, Ramos et al. 2003). The echinoderms *S. neumayeri*, *O. victoriae* and *O. validus* in this study had a factorial rise in oxygen consumption from winter to summer of 1.5, 1.4 and 1.5 respectively (Figure 4.18). Each one of these animals has the opportunity to feed all year round. *O. victoriae* and *O. validus* are opportunistic feeders and *S. neumayeri* feeds on a range of phyla. *O. validus* utilises a wide variety of available prey items and is reported to employ a range of feeding strategies including suspension feeding, grazing, scavenging and active predation (Pearse 1965). However their feeding levels decrease during the austral winter (Stanwell-Smith and Clarke 1998) which could explain the lower metabolic rates (Figure 4.14). In *O. validus* at Signy Island, the pyloric caeca index (food storage organ) increased in summer (typically November – March) and decreased during winter (June to October) suggesting that feeding activity was seasonal (Stanwell-Smith and Clarke 1998).

Gonad index values for this species in Signy Island (Stanwell-Smith and Clarke 1998) and Rothera (Grange, Tyler et al. 2007) peaked in April/May but larval numbers in the water column were high in August to October (Bowden, Clarke et al. 2009). This study is the first to present seasonal metabolic rate data for *O. validus*, therefore there is no interannual

comparison for this species. *O. validus* does however have interannual differences in reproduction and does not spawn every year. Oogenesis was described by Pearse (1965) and Grange, Tyler et al. (2007) as taking 18 to 24 months with overlapping generations of primary oocytes. Of my five common benthic invertebrates *O. validus* is the only one that spawns in winter, this may seem strange, but having larvae in the water column in winter reduces predation by suspension feeders such as *H. steineni*, which cease feeding in winter. The very slow development rates at low temperature also mean winter spawning is needed in some species for juveniles to be able to exploit the next summer's productivity. *O. validus* feed and build gonad during the austral summer. All these factors affect the differences observed in metabolic rates between summer and winter. The slope of the relationship between oxygen consumption and AFDM is the same in both summer and winter, with a mass scaling coefficient of 0.89 in both seasons. *O. validus* studied here require between 3 and 6 years before becoming mature and ready to spawn (Janosik and Halanych 2010). In this study, juveniles would have been utilising energy for somatic growth, and large adults for reproduction, but both appear to drive a similar seasonal change in metabolic rate.

Nacella concinna

The Antarctic limpet *N. concinna* has one of the lowest seasonal factorial changes in oxygen consumption (Figure 4.18). This is probably because it is reported to feed all year round (Fraser, Clarke et al. 2002) so there is less effect of seasonal variation of feeding on metabolic rates. Diatoms and filamentous algae dominate the diet of *N. concinna* (Segovia-Rivera and Valdivia 2016). The amount of food available to limpets is seasonal, as benthic biofilms rely on nutrient availability and light levels are seasonal, but photobionts are not the only components of benthic biofilms, and biofilms are less seasonal than the phytoplankton bloom and thus produce less metabolic seasonal variability in their consumers. Antarctic biofilms can have a large variability in community structure and biomass over short time scales, and there is the potential of different strategies in the biofilms to overcome fluctuating conditions (Gilbert 1991, Rochera,

Fernández-Valiente et al. 2013). Overall annual productivity can be higher in benthic sites than in the water column in ice dominated shallow sites in Antarctica (McMinn, Ashworth et al. 2012). This probably results in more even food availability throughout the year.

Metabolic rates in the larger limpets were higher in winter than in the summer months. The larger animals in this study were large reproductive adults and would not have been investing significant amounts of energy in somatic growth. These animals spawn in the summer at Rothera Point, in January and February (Peck 2016). The higher metabolic rate in winter is thus most likely because they were feeding and probably producing gonad ready to spawn in the following summer. Post prandial increases in metabolism (SDA) can last up to 15 days after feeding in Limpets (Peck and Veal 2001), and the experimental measurements here were taken between 2 and 5 days after the animals were collected. The smaller *N. concinna* had a higher metabolic rate in summer when these animals would have been investing energy in somatic growth.

Fraser et al. (2002) found metabolic rates decreased in winter in the limpet *N. concinna* but to a much lesser degree than other polar marine invertebrates. The metabolic rates for a limpet of 0.206g AFDM in the summer of 2015 in this study were significantly higher at $0.98 \mu\text{mol O}_2 \text{ hr}^{-1}$ than those measured by Fraser et al. (2002) of $0.61 \mu\text{mol O}_2 \text{ hr}^{-1}$, the 95% confidence limits being between 0.864 and 1.104 (Figure 4.17). A limpet of 0.206g AFDM (using the regression equation from Fraser 1999, unpublished data is 21mm length), would be considered mature and would be 7 – 8yrs old. *N. concinna* is a broadcast spawner with pelagic larvae (Peck 2016). Spawning occurs in a narrow time frame and after the sea water temperature has warmed up to above -0.5°C (Stanwell - Smith et al. 1998). The summer measurements of metabolic rates in 2002 were taken in December prior to the sea temperature reaching its peak and before the limpets spawning period, whereas the 2015 data were collected between January and March during the spawning period (some animals were observed spawning in the aquarium) which might explain

the higher summer metabolic rates in this study. Figure 4.19 also shows a significantly higher metabolic rate in *N. concinna* during the austral winter of 2015 compared with 2002. The winter of 2002 had 208 days of fast ice whereas 2015 had 124 days. This means in 2015 there would have been more light penetration to the sites where *N. concinna* is found that would likely have increased the quantity and quality of the benthic biofilms and therefore more grazing opportunities for the limpet.

Obermüller, Morley et al. (2010) also measured seasonal metabolic rates in *N. concinna* in the winter of 2008 and summer of 2009. The animal size range used by Obermüller, Morley et al. (2010) was smaller than Fraser et al. (2004) and hence the comparison made here was on a standard animal of 0.148g AFDM. On this basis, the metabolic rate for *N. concinna* in this study in the austral summer of 2015 was significantly lower than the data collected in the austral summer 2009 (Figure 4.19). In the summer of 2009 the metabolic rate was $1.04 \mu\text{mol O}_2 \text{ hr}^{-1}$ compared with $0.75 \mu\text{mol O}_2 \text{ hr}^{-1}$ in 2015, and this was significantly different (95% confidence interval = 0.65 - 0.84).

The animals used in 2009 were intertidal limpets which may expend more energy (higher metabolic rate) than sub-tidal limpets (Weihe and Abele 2008) due to increased shell thickness (Hoffman, Peck et al. 2009), shell damage repair from ice impacts (Cadée 1999) increased shell height (Morley, Belchier et al. 2014) increased tenacity (Davenport 1988) and increased predation pressure from Kelp gulls and Skuas (Branch 1984, Davenport 1997). In winter, once the sea has frozen and the sea birds have migrated north, many of these factors are removed which may explain why the metabolic rates are not significantly different between intertidal 2008 and sub-tidal 2015 winters (Figure 4.19), although other factors such as ice cover duration and timing of measurement could also be having an effect. In the winter of 2008 the metabolic rate was $0.84 \mu\text{mol O}_2 \text{ hr}^{-1}$ compared with winter 2015 that was 0.77 and these were not significantly different (95% CI's overlap).

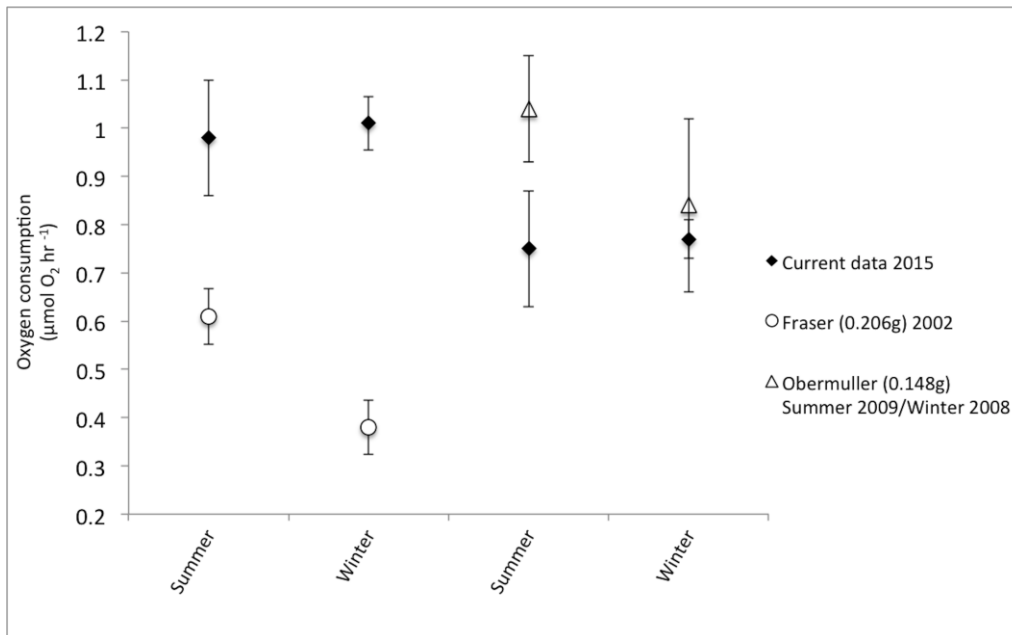


Figure 4.19 Oxygen consumption in *Nacella concinna* between seasons and years for a mass corrected animal \pm SE. Data were corrected to a standard animal of 0.206g AFDM to compare with Fraser et al. (2002) and 0.148g AFDM to compare with Obermüller et al. (2010).

Ophionotus victoriae

O. victoriae had a metabolic scaling coefficient of 0.78 ± 0.6 , which is not significantly different from 0.75 (ANOVA $F_{(1,55)} = 0.37$ $P=0.55$), the expected value from the metabolic theory of ecology (Gillooly, Brown et al. 2001, Kleiber 2001). The minimum disc size for a mature *O. victoriae* is 11.2mm (Grange et al. 2004) and using a regression of disc diameter to AFDM (Vausse, B pers comms) this would equate to an AFDM of 0.074g. In the present study all animals used (except one in winter) would have been sexually mature. The spawning period of this species at Rothera Point is in November and December (Grange, Tyler et al. 2004). The summer metabolic measurements for *O. victoriae* in this study were made between January and March, which is therefore likely to be post spawning and could help explain the lack of significant difference in metabolic rate between summer and winter.

Obermüller et al. (2010) reported a metabolic rate for *O. victoriae* in the summer of 2007/2008 of $4.0 \text{ O}_2 \text{ h}^{-1} \text{ g}^{-1} \text{ AFDM}$ which, was higher than this study ($3.22 \text{ O}_2 \text{ h}^{-1} \text{ g}^{-1} \text{ AFDM}$), and the difference was significant (95% confidence intervals 2.74 - 3.70 Table 4.3). This could be due to some measurements in 07/08 being taken during spawning as the summer measurements were between November and May. Some care needs to be taken when comparing metabolic rates per gram of animal tissue as mass specific physiological rates vary with size. The more accurate comparison would be for a standard animal. For example large individuals have a higher metabolic rate than small animals, but the scaling coefficient of 0.75 means their per gram values will be lower than measurements on smaller individuals.

Obermüller et al. 2010	Obermüller et al. 2010	This study 2015	This study 2015
Summer 2007/2008	Winter 2008	Summer 2014/2015	Winter 2015
November – May	June - October	January - March	June – October
$4.0 \text{ O}_2 \text{ h}^{-1} \text{ g}^{-1}$	$1.95 \text{ O}_2 \text{ h}^{-1} \text{ g}^{-1}$	$3.22 \text{ O}_2 \text{ h}^{-1} \text{ g}^{-1}$	$2.50 \text{ O}_2 \text{ h}^{-1} \text{ g}^{-1}$
0.84 SE	0.53 SE	0.23 SE	0.11 SE
N = 12	N = 12	N = 22	N = 34

Table 4.3 Interannual and seasonal metabolic rate comparison for *O. victoriae* based on mean oxygen consumed per gram (AFDM) of animal tissue.

There is a connection between the sedimentation events at Rothera and ophiuroid reproductive characteristics (Grange, Tyler et al. 2004). The magnitude and duration of each annual sedimentation event may play an important role in the following years reproductive success. Patterns of ice cover and thickness probably modulate sedimentation, which then underpin reproductive output the following year (Grange, Tyler et al. 2004). The winter of 2006 had 160 fast ice days, which would have

allowed a large amount of ice algae to become established and producing significant sedimentation when the ice broke out. In the 2013 winter there was less than half the number of fast ice days compared with 2006 (70 in total). This would have meant less sedimentation and therefore less food for *O. victoriae*, which could help to explain the lower metabolic values in 2014/15 than 2007.

O. victoriae has a slow growth rate and can live up to 22 years (Moya, Ramos et al. 2003), and growth in ophiuroids is highly variable. Environmental and internal factors such as temperature, food supply or reproduction can trigger changes in skeletal growth (Dahm and Brey 1998). As growth and reproduction depend on food availability, which depends on sea ice duration and breakout, it is not surprising to see metabolic variation between years.

Heterocucumis steineni

Of the five common benthic marine invertebrates chosen for seasonal metabolic rate analysis the holothurian *H. steineni* is the only obligate primary consumer and filter feeder. *H. steineni* also had the largest factorial change in oxygen consumption between summer and winter out of the five species in this study (Figure 4.18).

In the current study I attempted to assess the metabolic needs across the whole population, which is why I measured metabolic rates across a wide size range of *H. steineni*. Over a size range of 0.17g AFDM – 14.77g AFDM there was no significant seasonal difference (GLM $F_{(1,55)} F = 2.47, P = 0.122$), which is surprising considering *H. steineni* relies on the seasonal summer phytoplankton bloom for food. There was, however, a significant difference in the rate of change in metabolic rates with AFDM (slope of the relationship in Fig. 4.13) between seasons (GLM $F_{(1,55)} F = 7.00, P = 0.011$). Caution needs to be taken interpreting these data as the majority of the points are large individuals and there are only a few measurements on juveniles.

There appears to be some evidence of a metabolic difference in the larger individuals between seasons. *H. steineni* spawns in late December which, as it also coincides with the largest period of food supply, should produce higher summer metabolic rates. Comparing metabolic rates for a standard animal of 0.3g AFDM, which is a mature adult, there is a significant seasonal difference (T test $T = 6.39$, $P < 0.001$). The data for *H. steineni* also shows interannual variation when compared with Fraser (1999 published in Fraser et al. 2004) as measured routine oxygen consumption is 30% to 80% higher in this study than in 1999 (Figure 4.20). Polar reproductive cycles typically have a long duration and gametogenesis (Grange, Tyler et al. 2004). Interannual variation in food availability may lead to a difference in reproductive effort between years. As with *S. neumayeri*, *H. steineni* shows higher metabolic rates in both seasons compared with 1999 (Figures 4.16 and 4.20). It has been observed that during the phytoplankton bloom 70 – 100% of *H. steineni* will be feeding at any one time (Fraser, Peck et al. 2004).

A previous study on the sea urchin *S. neumayeri* demonstrated that seasonal fluctuations in water temperature only account for 15-20% of the variation in seasonal respiration, while increased physiological activity (feeding, growing and spawning) driven by increased food consumption account for the remaining 80-85% (Brockington, Clarke et al. 2001). In winter, when there is no food available for filter feeders, *H. steineni* has been reported to enter a hypometabolic state similar to hibernation (Fraser, Peck et al. 2004), and will often not be visible unless rocks are moved. The data here, with only large individuals exhibiting variation in metabolic rate suggest reproductive costs caused this seasonality. The lack of seasonality in metabolism across the size range differs from the conclusions drawn by Fraser, Peck et al. (2004) and might be due to the much smaller phytoplankton bloom in 2015 compared to 2003 (Figure 4.17).

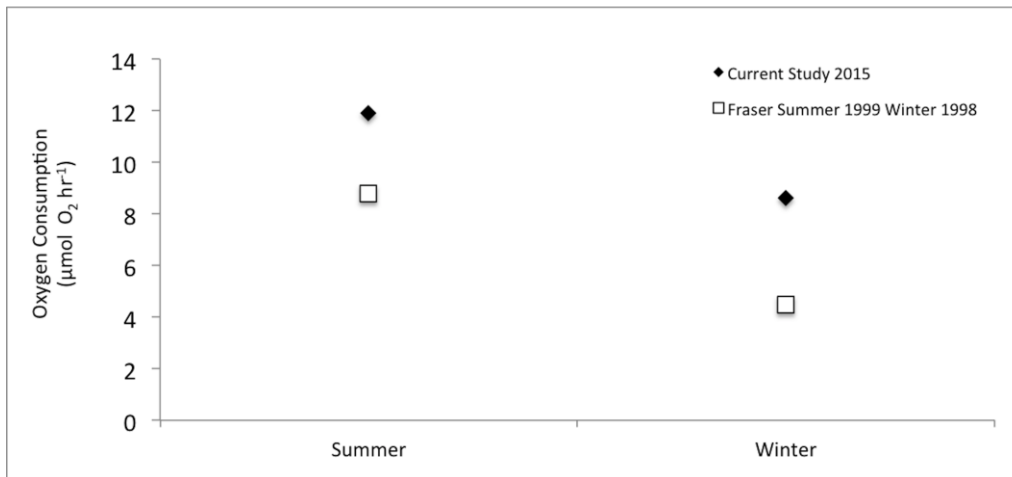


Figure 4.20 Oxygen consumption for *H. steineni* between seasons and years for a mass corrected animal of 7.5g AFDM for summer and 8.0g AFDM for winter. Data from summer (February 1999) and winter (July 1999) and in this study summer (Jan- March 2015 N = 25), winter (June - October N = 31).

4.5 Conclusions

The hypothesis that seasonal change in metabolic rates will vary differently between animals of different trophic levels, and that secondary consumers would be less affected by seasonality than primary consumers is not supported by my data, although a caveat is there was only one primary consumer in the study. Metabolic rates varied between species but there was no clear-cut difference between my primary consumer and the secondary consumers. The secondary consumers *O. validus* and *O. victoriae* showed the same factorial increase in oxygen consumption from winter to summer as the primary consumer *H. steineni*. The variability of seasonality of metabolism within this study was typical of Antarctic marine benthic invertebrates, and would be expected due to the diversity of ecological roles of organisms studied and the associated energy demands within those roles.

This study has enabled inter-annual comparisons of metabolic rates in *O. victoriae*, *H. steineni*, *N. concinna* and *S. neumayeri* which is the first such comparison for these Antarctic marine species. However, to be able to draw solid conclusions on inter-annual effects and quantify the scale of inter-annual variation more research in different years is needed. The data

in this study do, however, give some level of confidence in our assessments of changes in metabolic rates between seasons as, where previous studies have been conducted the level of change is similar between years. This study also shows Antarctic secondary consumers have much more seasonally variable metabolic rates than would be expected given a year round food supply and this may be an effect of food quality varying seasonally, or the requirement for food being seasonal due to some other factor than a direct impact of food availability.

While food availability is the obvious main driver in the effect of season on metabolic rates within species there are other variables (as discussed above) to consider that could explain the diversity of results within the different trophic levels. Irrespective of the causes there is strong seasonality in the metabolism of the vast majority of Antarctic benthic marine invertebrates investigated whether primary or secondary consumers.

Chapter 5 – The use of molecular
methods for determining the diet of
marine invertebrates: preliminary
investigations

Chapter 5 The use of molecular methods for determining the diet of marine invertebrates: preliminary investigations

5.1 Introduction

Understanding the dietary habits of marine invertebrates is central to studies of food webs, ecological processes, energetics and natural history. Hence the nature of trophic interactions is a fundamental question in Ecology and has commanded the attention of biologists for decades (Shehzad, Riaz et al. 2012). Predator–prey interactions are amongst the main processes controlling change in animal populations and thus are central to many ecological studies (Symondson 2002). Marine invertebrates make up a huge percentage of the oceans biodiversity, yet the diets of most marine invertebrates are poorly known (Blankenship and Yayanos 2005). The diet of an organism may change depending on supply and season. This is particularly so in the case of polar animals in which experience intense seasonality (Clarke 1988, Clarke, Meredith et al. 2008). Mohan, Connelly et al. (2016) noted that there were different feeding modes among Arctic marine invertebrates depending on season and many Antarctic benthic marine invertebrates also exhibit seasonal cycles in the feeding activity (Brockington, Clarke et al. 2001, Fraser, Clarke et al. 2002, Fraser, Peck et al. 2004, Morley, Peck et al. 2007). In contrast, some Antarctic species such as the bryozoan *Arachnopusia inchoata* feed all year round (Barnes and Clarke 1995).

There is a cost to feeding; species may not feed even when food is present. The benefits of feeding would depend on both quality and quantity of available food, and both are already being affected by climate change. For example, the magnitude of the spring phytoplankton bloom is much reduced following winters with reduced sea-ice cover, clearly impacting filter feeders (Venables, Clarke et al. 2013).

However reduced sea ice cover also increases the duration of the phytoplankton bloom, so even though the magnitude of the bloom may be lower, the longer duration means a longer period of feeding and therefore potentially more growth. Whether this is occurring in Antarctica has yet to be determined. Also, the frequency of ice scouring is highly seasonal and changing (Smale, Barnes et al. 2006, Barnes and Souster 2011).

Disturbance events may cease entirely during winter whilst icebergs are 'locked in' by seasonal fast ice which, means that the reduction in ice-mediated disturbance (and therefore faunal mortality) during winter is likely to result in a reduction in feeding opportunities for scavengers.

However, the period of winter fast ice is decreasing (Ducklow, Fraser et al. 2013) and so food supplies for scavengers should increase in the future (Smale, Barnes et al. 2007). Additionally, sea temperatures are increasing, which will directly impact species metabolic rates. Peck, Webb et al. (2008) showed that there is a loss of feeding competence (SDA, which is the amount of energy expenditure above the resting metabolic rate due to the cost of processing food for use and storage) with an increase in temperature for Antarctic marine invertebrates. It is therefore important to understand species diets now, so if seawater temperatures increase in future we can understand the subsequent effects on different trophic levels and therefore the Antarctic ecosystems.

Traditional diet analyses mostly rely upon the morphological identification of undigested remains in the faeces (Shehzad, Riaz et al. 2012) or in the gut (Peck, Barnes et al. 2005, Vannier 2012). However prey species with robust hard parts, which can readily survive digestion, are likely to be over-represented in such analyses. Whereas prey species with less robust or no hard parts are likely to be under-represented, or not represented at all. Not all predators ingest such hard remains even if present in the prey, and even those that do consume them may also ingest soft-bodied prey that leave no recognizable remnants (Symondson 2002). With these limitations in mind, new technologies and the use of genetic techniques based on polymerase chain reaction (PCR) amplification of DNA from gut contents (prey items) have been successfully applied for

marine vertebrates (Deagle, Jarman et al. 2005, Deagle, Gales et al. 2007, Deagle, Kirkwood et al. 2009) and invertebrates (Blankenship and Yayanos 2005, Deagle, Jarman et al. 2005, Hu, Guo et al. 2014, Mohan, Connelly et al. 2016). That said work with Antarctic species is still underdeveloped.

The original intention of this chapter was to investigate the seasonal variation in the diet of the five common benthic marine invertebrates used to study seasonal variation of metabolism in Chapter 4; the limpet *Nacella concinna*, the cushion star *Odontaster validus*, the brittle star *Ophionotus victoriae*, the sea cucumber *Heterocucumis steineni* and the urchin *Stereochinus neumayeri*. These species are important components of the ecosystem, are significant energy transformers, and employ a range of feeding strategies. Reports to date suggest that *H. steineni* is a primary consumer and suspension feeder (Fraser, Peck et al. 2004), *S. neumayeri* is an omnivorous benthic pioneer species where a large part of their diet comes from scavenging (McClintock 1994), *N. concinna* feeds all year round and the diet consists mostly of diatoms and filamentous algae (Fraser, Clarke et al. 2002) and the echinoderms *Ophionotus victoriae* and *Odontaster validus* have catholic diets and demonstrate a range of feeding strategies, including opportunistic scavenging and cannibalism (McClintock, Pearse et al. 1988, McClintock 1994). *O. validus*, *S. neumayeri*, *N. concinna*, *O. victoriae* all have teeth and *O. validus* also performs external digestion.

Given these different feeding strategies, morphological identification of prey items is problematical and many prey species could remain unaccounted for. Coupled with the fact that molecular techniques for identifying digested and macerated prey items are available but have not yet been optimized for the five species studied here, the original study aim had to be changed. The revised aim of this chapter therefore was to make a start to testing and validating, molecular techniques for identifying prey items of the five chosen Antarctic species. I did this by first amplifying (PCR) and sequencing DNA from each of the five host species.

Unfortunately due to time constraints I was only able to amplify and sequence DNA from the gut and faecal material of only one of the species *H. steineni*. This molecular identification prey was then compared with light and SEM microscopy identification of prey morphospecies in an attempt to validate the technique.

5.2 Methods

Sixteen *O. validus*, *S. neumayeri*, *O. victoriae*, *N. concinna* and *H. steineni* were collected during the austral summer (November – April) by hand using SCUBA and another sixteen of each in the austral winter (June – October) from depths between 6m and 20m and between the location of Cheshire Island and South Cove just south of Rothera Research Station (Figures 1.2b and 2.1, Chapters 1 and 2 respectively). After collection, specimens were returned to the Bonner Laboratory with care being taken to ensure they remained submerged at all times. They were held individually in beakers in a flow through aquarium as described in Chapter 4. Within 4 hours of collection they were dissected, their stomachs removed (Figures 5.1 - 5.5) and contents extracted. Gloves were used to avoid contamination. Any faeces in the temporary storage beakers were also collected using a pipette.

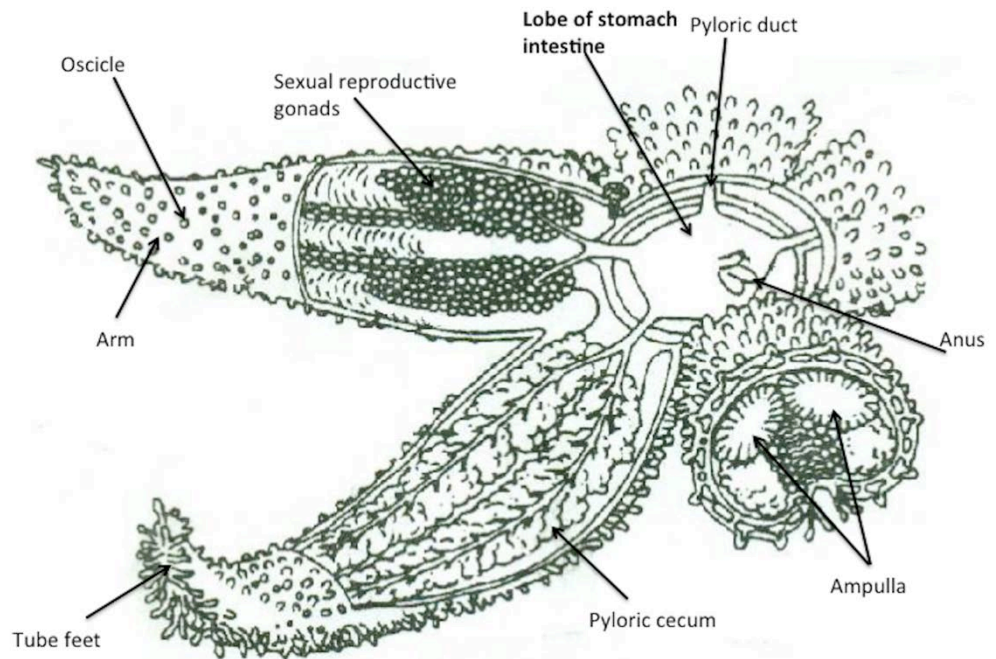


Figure 5.1 Dissection diagram showing the location of the gut in a generalised starfish (representing *O. validus* (Davis 2012)).

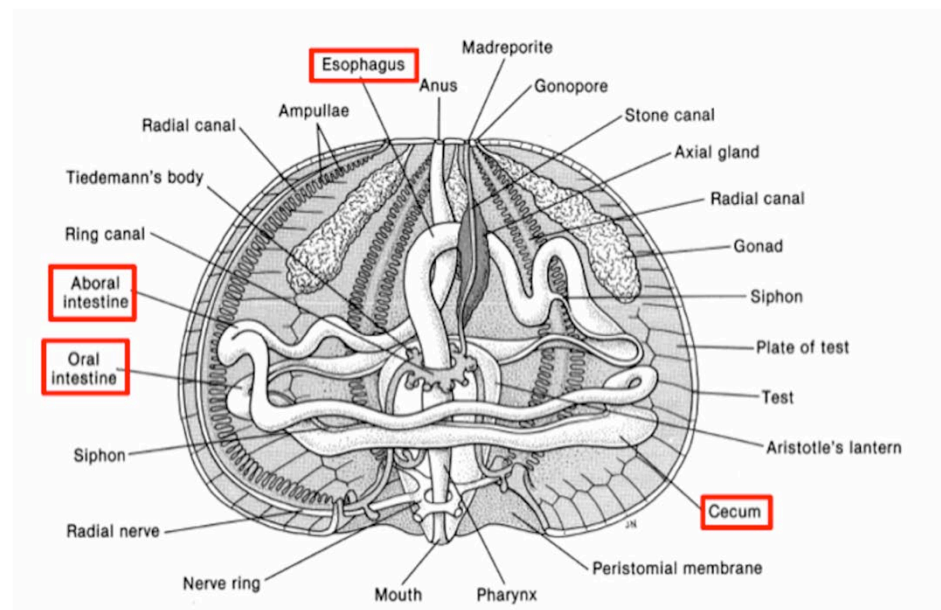


Figure 5.2 Dissection diagram showing the location of the gut (oesophagus and intestine) in a generalised sea urchin (representing *S. neumayeri* (Whalen 2008)).

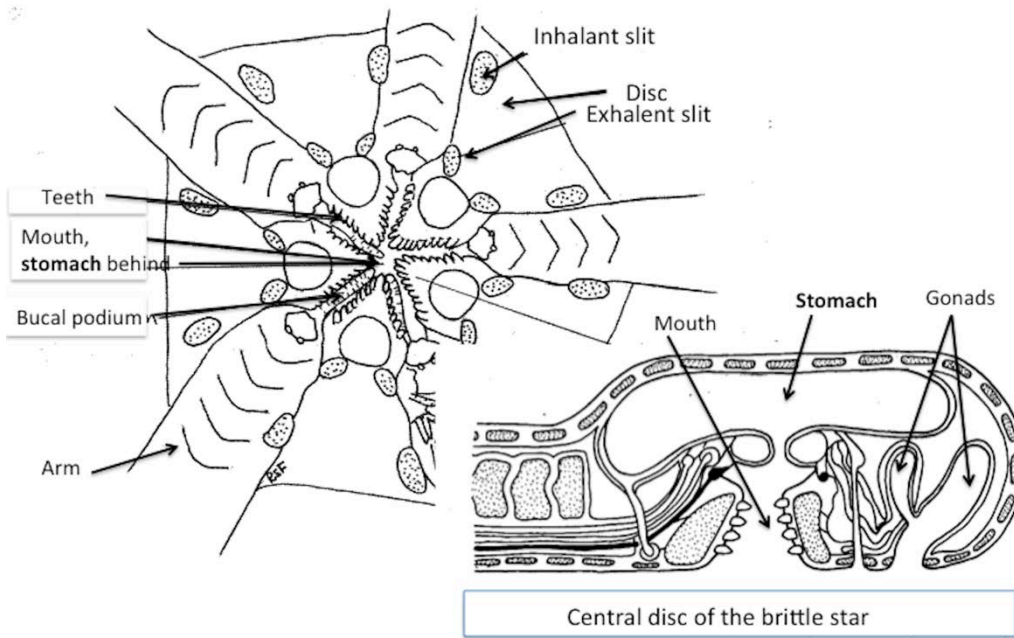


Figure 5.3 Dissection diagram showing the location of the gut (stomach) in a generalised brittlestar (representing *O. victoriae* (O'Brien 2006, Fox 2007))

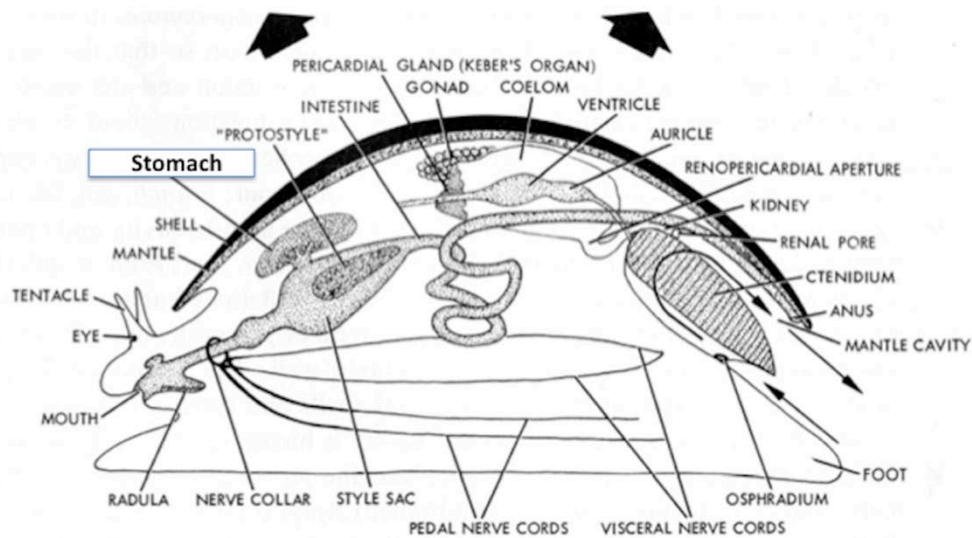


Figure 5.4 Dissection diagram showing the location of the gut (stomach) in a generalised limpet (representing *N. concinna* (Sherman and Sherman 1976)).

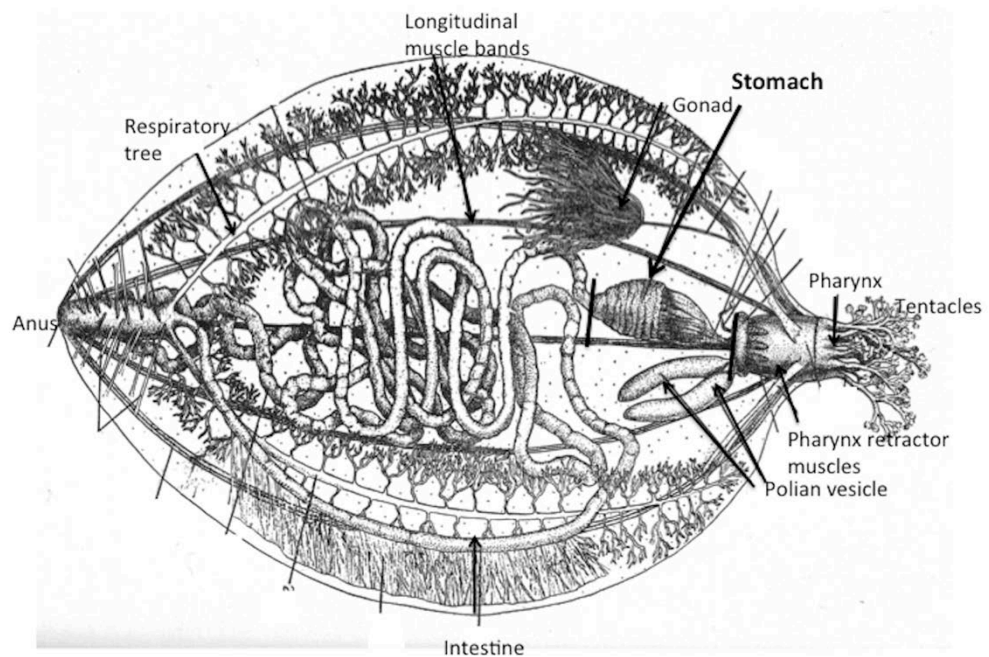


Figure 5.5 Dissection diagram showing the location of the gut (stomach) in a generalised sea cucumber (representing *H. steineni* (Fox 2007)).

Gut contents from *H. steineni* were extracted using a scalpel, scraping away the obvious brown content (presumed phytoplankton) inside the stomach. The stomach of *N. concinna* was removed and the contents aspirated into a pipette. The stomachs for *O. victoriae*, *S. neumayeri* and *O. validus* were removed and macerated. All contents of each gut and each species were placed into plastic vials and frozen at $T = -20^{\circ}\text{C}$. Similarly any faeces collected were stored in separate vials.

DNA Extraction

DNA extractions took place in a UV sterilised laminar flow hood. The gut contents were defrosted at room temperature and the DNA extracted using sterilised tools and the Bio Ultra Clean DNeasy DNA extraction kit according to manufacturers instructions. To estimate how much DNA had been extracted a biophotometer (uses ultra violet light to measure amount of DNA in sample) was used with DEPC treated water (sterile water which is suitable for use with DNA and RNA by incubating with 0.1% diethylpyrocarbonate and then autoclaved to remove DEPC) as the blank reading.

PCR amplification of host DNA

Whilst a number of different “universal” primer sets are recorded in the literature for amplification in barcoding studies, they do not all work with the same efficiency across species. Due to the gut contents of the species being largely unknown and the vast majority of prey items lacking suitable DNA identifying barcodes, an initial experiment was conducted to identify a universal primer set which consistently produced a PCR product of the correct size across all species and was conducted using the five host species as the test species.

DNA extracted from the hosts *O. validus*, *S. neumayeri*, *O. victoriae*, *N. concinna* and *H. steineni* were diluted 1:10 and then amplified using 2 pairs of 18S, 2 pairs of 16S and one pair of CO1 gene primers as identified from the literature (Blankenship and Yayanos 2005) (Table 5.1).

A	18S	NSF4 NSR581	CTGGTTGATYCTGCCAGT ATTACCGCGGCTGCTGGC
B	18S	NSR1419/20 NSR1642/16	AGCATAACAGGTCTGTGATGCCC GACGGGCGGTGTGTRC
C	CO1	LC0I1490 HCO2198	GGTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA
D	16S	16Sa 16Sb	AACCTGGTTGATCCTGCCAGT TGATCCTTCTGCAGGTTACCTAC
E	16S	16SAR 16Sbr	CGCCTGTTTATCAAAAACAT CCGGTCTGAACTCAGATCACGT

Table 5.1 The five potential sets of primer pairs tested for the amplification of prey items from the guts of Antarctic marine invertebrates.

The PCR cycle used varied with the primer pairs, as follows.

18S from biofouling manuscript (Peck, Clark et al. 2015)

94°C 30s

then 35 cycles of:

94°C 30s

55°C 30s

72°C 60s

Final extension of 72°C for 5 min

The 18S PCR cycle was subsequently modified slightly with an increase in annealing temperature to 58°C.

16S (Webb, Barnes et al. 2006)

95°C 5 min

then 40 cycles of:

94°C 30s

52°C 30s

72°C 60s

Final extension of 72°C for 7 min.

C01 (Blankenship and Yayanos 2005)

95°C 15 min

80°C 5 min

then 40 cycles of:

92°C 90s

42°C 60s

Final extension of 72°C for 2 min.

CO1 (Webb, Barnes et al. 2006)

94°C 4 min

then 4 cycles of:

94°C 1 min

45°C 90s

72°C 90s

then 35 cycles of:

94°C 60s

50°C 90s

50°C 90s

72°C 1 min

Final extension of 72°C for 5 min.

The PCR was carried out using Bioline Reagents (Table 5.2)

	1 reaction (µl)
10x buffer	5
dTNP mix	5
Primer 1 NSF4 (10mM)	2
Primer 2 NSR581 (10mM)	2
MgCl ₂	1.5
BSA	1
Water	33.25
Taq	0.25
Sample DNA	1
Total volume	50

Table 5.2 PCR, a technique used in molecular biology to amplify a single copy or copies of DNA across several orders of magnitude. This table shows the reagents used in the master mix to amplify gut content DNA.

The PCR amplification was then run using gel electrophoresis to check that the amplification of DNA had worked. To obtain a high quality sequence for future analysis, the PCR products were subcloned into the pGEM-TEasy vector (Promega) prior to Sanger sequencing (at Source Bioscience) as follows:

Sub-cloning and bacterial transformation

The PCR product was purified using the QIAquick PCR purification kit (Qiagen) following manufacturer's instructions and eluted in 30ul EB buffer. The PCR product was then ligated into a cloning vector at 4°C overnight (pGEM-TEasy (Promega) (Table 5.3).

Vector (50mg ul ⁻¹)	1µl
10x ligation buffer	1µl
T ₄ DNA ligase (temperature sensitive)	1µl
PCR product	7µl

Table 5.3 Reagents and their volumes used in the ligations of the PCR products

The ligation mix was then transformed into DH5a cells (Invitrogen, ThermoFisher) to obtain single colonies for PCR amplification and sequencing.

TYE media plates for bacterial transformation

TYE media (Table 5.4) was melted in the microwave and then cooled to approximately 50°C where the amphibiotic ampicillin was added (100µl ampicillin (50mg ml⁻¹ stock solution) per 100ml media). The medium was then poured and shared over a number of 14cm clear plastic plates and left to set in a class II cabinet with the lids slightly off. Once set, these plates were stored in the fridge ready for use.

SOB Media 20g Tryptone 5g yeast extract 0.5g NaCl Make up to 990ml final volume Add 10 ml 250 mM KCl	SOC Media 10ml of SOB into 15ml flacom tube Add 100µl 1M MgCl ₂ Add 100µl 1M MgSO ₄ Add 100µl 20% glucose
2 x TY 16g tryptone 10g yeast extract 5g NaCl Make up to 1l and autoclave	Agar media Add 15g agar to 1l of 2xTY

Table 5.4 Media required for sub cloning and bacterial transformations.

Bacterial transformation

The bacterial cells, *E. coli* were thawed on ice. 50µl of the cells were added to a 1.5 ml microcentrifuge tube (Eppendorf) along with 1µl of the ligation mix and left on ice for 10 -30 min. The cells were then heat shocked at 42°C for 20 sec only and placed straight back on ice. 500µl of SOC (Table 5.4) prewarmed to 37°C was added to each tube and incubated with shaking for 1 hour. While the cells where incubating, the TYE ampicillin plates were warmed by incubating at T = 37°C. 25µl of X-Gal (Bioline) was then added to bacterial solutions and the transformation mix was spread over the surface of the TYE plates and left to soak in. Once the liquid had soaked into the agar, the plates where inverted and incubated overnight at T = 37°C.

Colonies that contained recombinant clones were selected by X-gal-mediated blue/white selection where white colonies contained the DNA insert. A selection of white colonies were picked into 100µl TYE media in a 96 well plate. PCR amplification of each clone was carried out and run on an agarose gel to check for successful DNA amplification. The PCR products were then sent to Source Bioscience for Sanger sequencing to obtain the insert DNA sequences.

PCR amplification of gut contents using 18S NSF4 and NSR581 primers

DNA from 160 gut content samples was frozen (16 from the summer and 16 from the winter) of each of the host species. Ten samples of gut DNA were defrosted at a time due to the use of a gel containing twelve wells. The PCR cycle used was: 94°C 30s then 35 cycles of 94°C 30s - 58°C 30s - 72°C 60s and finally 72°C for 5 min. The PCR amplification was then checked on gel electrophoresis and successful amplifications were then frozen at -20°C. As the PCR (predator DNA) contained multiple species from within the gut (prey) as well as host (predator DNA), the PCR product was digested with a host specific restriction enzyme.

Restriction enzyme digestion of 18S PCR products

To remove the host DNA, restriction enzyme digestion was used. Restriction enzyme maps of the host 18S PCR products amplified and sequenced previously in the primer testing (detailed above) were produced using the remap programme (<http://emboss.bioinformatics.nl/cgi-bin/emboss/remap>) and appropriate enzymes chosen.

The original gut and faeces samples of one *H. steineni* were aliquoted into four microcentrifuge tubes and amplified using the 18S primers (NSF4 NSR581 Table 5.1). The PCR products were cleaned up using QIAquick PCR purification kit (Qiagen) following manufacturers instructions. Three restriction enzymes (PstI, PvuI and StuI (NEB)), which cut the host DNA at different regions, were trialled and one control was used with no enzyme incubation. The restriction enzymes were added to the PCR product in a

10µl reaction volume and incubated overnight at 37°C, following manufacturers instructions. The PCR products were then run on an agarose gel stained with Gel Green. This was to enable the excision and purification of an intact DNA band which should have contained mainly prey DNA. A control 18S PCR product with no restriction enzyme was run alongside the digested products to facilitate identification of where the uncut (i.e. prey) DNA was positioned in the Gel Green agarose gel. The appropriate Gel Green band was cut out and the DNA extracted from the gel at the position of the gel using the Qiagen gel purification kit according to manufacturers instructions. The previous steps were then followed of ligation and bacterial transformation (Figure 5.6). Up to 12 white colonies from each transformation were selected for sequencing.

Summary of molecular methodology

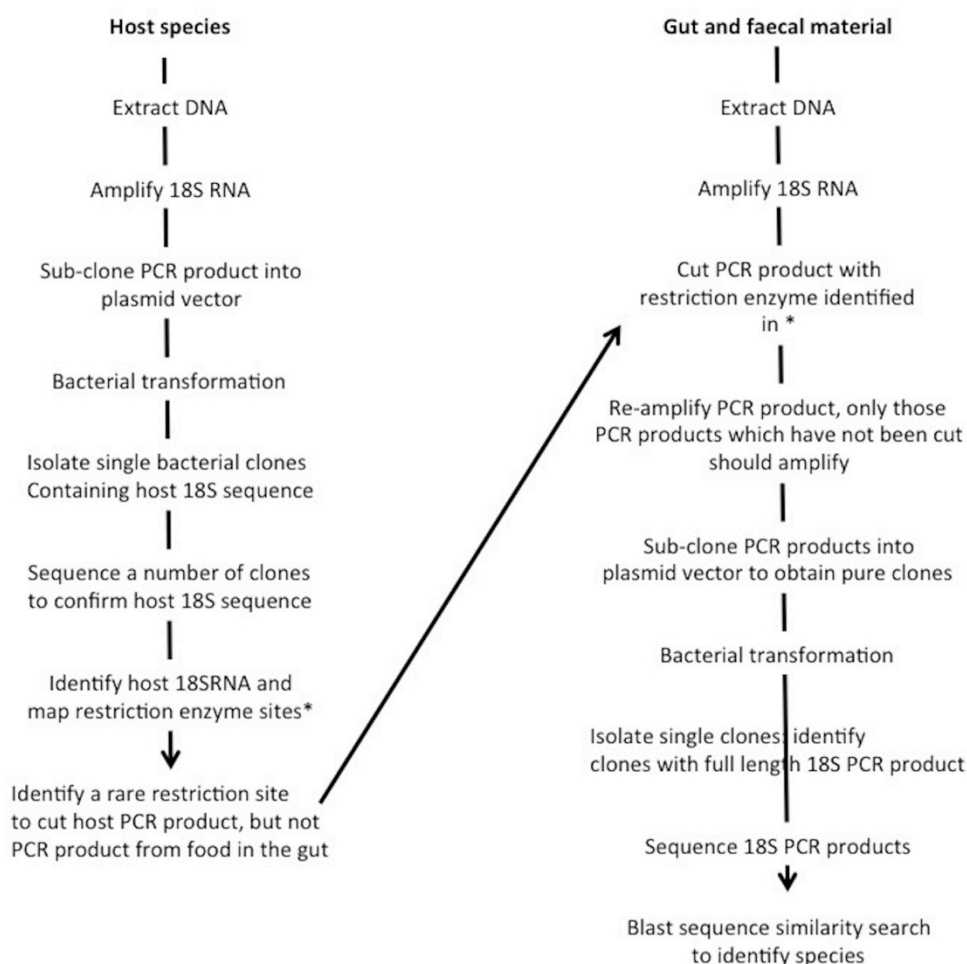


Figure 5.6 Diagram showing the method used for obtaining the DNA of prey items

Morphospecies identification for validation of molecular results

Light microscopy

Gut contents of four different *H. steineni* were placed on four different glass microscope slides. Ethanol (96%) was used as a preservative and to dilute the contents making identification of prey items clearer.

Photographs of the gut contents, i.e. prey items (Figure 5.7) were taken with a Nikon D7000 and identified (Table 5.5) by a Southern Ocean phytoplankton and diatom expert Dr Jaqueline Stefels, University of Groningen.

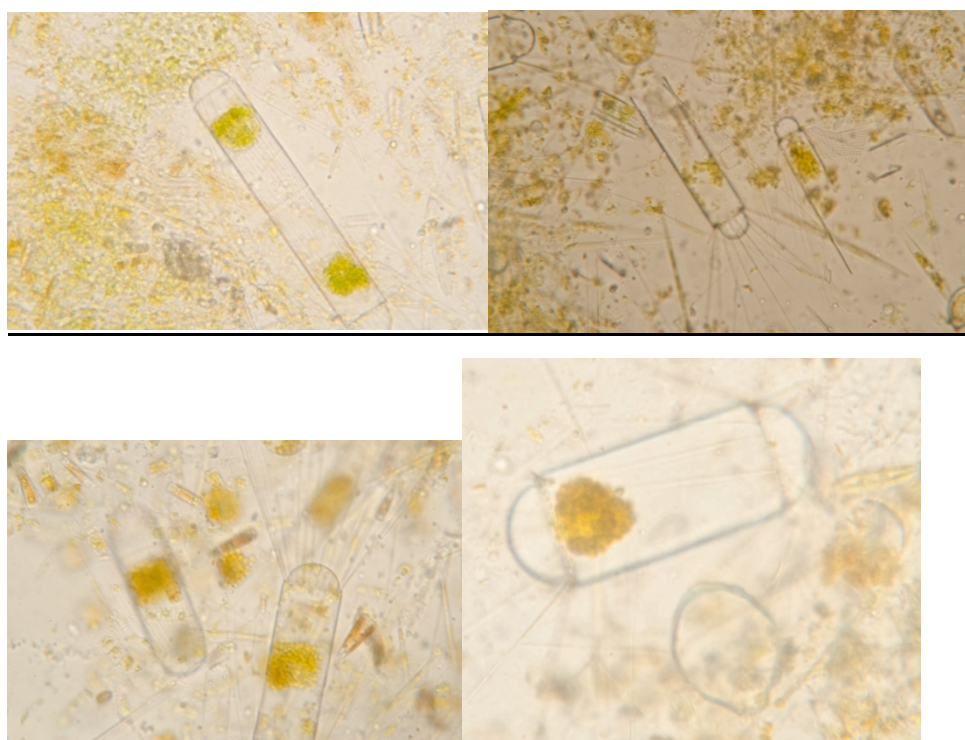
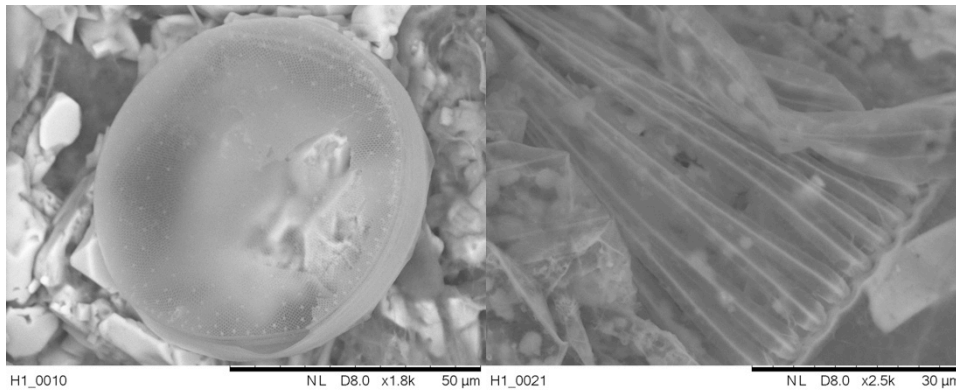


Figure 5.7 Light microscope images x50 magnification of *Corethron* sp of diatom found within the gut contents of *H. steineni*

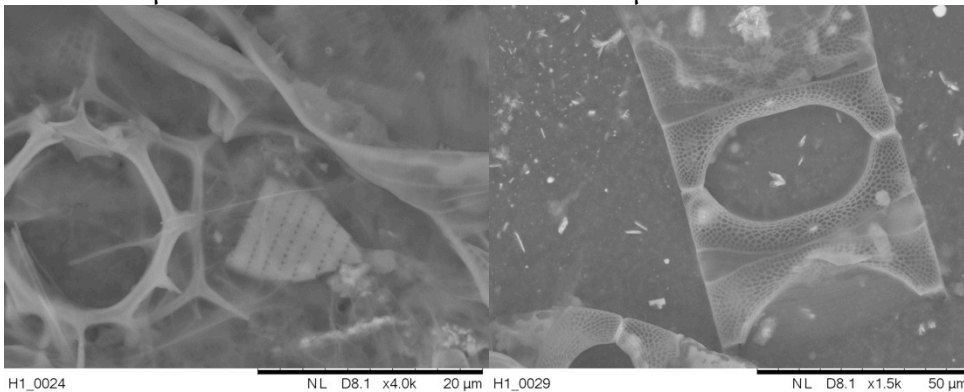
Scanning electron microscopy (SEM)

Gut contents for three *H. steineni* were scraped onto a scanning electron microscope (SEM) stub and analysed using an SEM. The pictures of the prey items (Figure 5.8) were then identified by Dr Claire Allen, an expert in Southern Ocean diatoms, British Antarctic Survey.



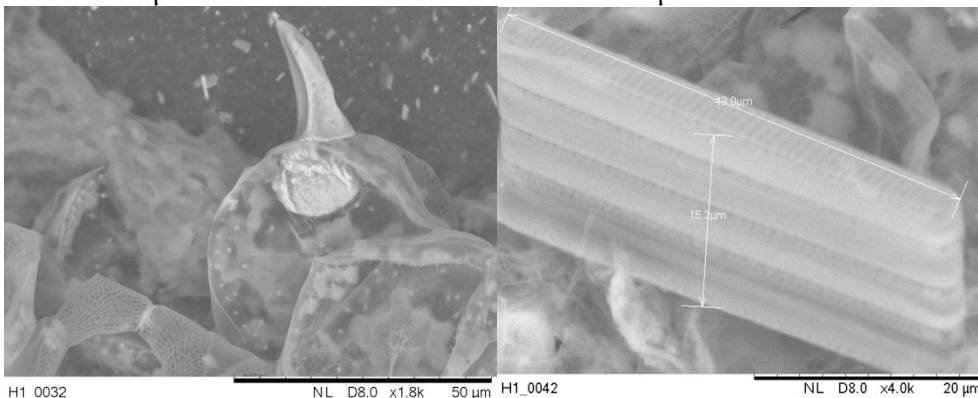
Porosira pseudodenticulata
8 x 1.8k 50µm

Corethron sp
8 x 2.5k 30µm



Left – Silicoflagellate,
Dictyocha speculum
8.1 x 4k 20µm

Eucampia antarctica var *recta*
(Vegetative)
8.1 x 1.5k 50µm



Thalassiosira gravida
8 x 1.8k 50µm

Colony of *Fragilariopsis* sp
8 x 4k 20µm

Figure 5.8 SEM images of some of the diatoms from the gut contents of *H. steineni*

5.3 Results

Molecular results

Both sets of 18S primers programmes amplified product in of the 4 species (see Appendix 2.1 for genetic barcodes of the host species) when the DNA was diluted 1:10, but not *H. steineni*. However, amplification was achieved with *H. steineni* when the DNA was diluted to 1:30 (Figures 5.10 and 5.11). Of the 2 x 18S primer sets (Table 5.1), Primer set B amplified a very small 200bp product and was discarded. The amplification with the 16Sprimers was more variable; primer set D (Table 5.1) 2 species were amplified, primer set E amplified 3 species. The COI performed the least efficiently and only amplified 2 species relatively cleanly with the Blankenship and Yayanos (2005) PCR program, the Webb, Barnes et al. (2006) program amplified 1 species and multiple bands for 2 others. *S. neumayeri* amplified well on the 18S, 16S and CO1 primers. Thus, the 18S primers NSF4 and NSR581 were chosen for all future analysis.

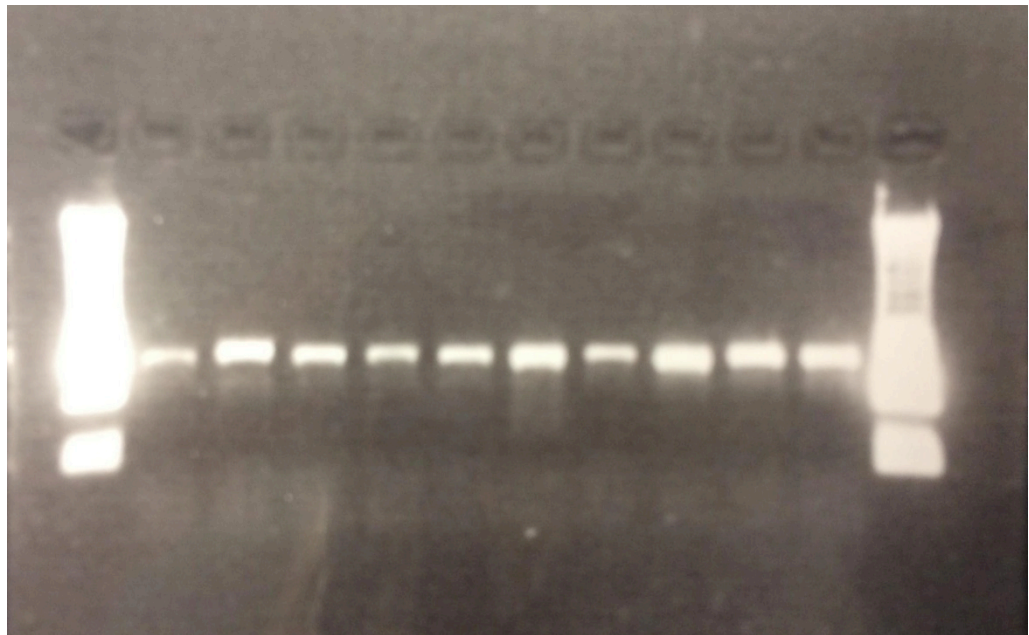


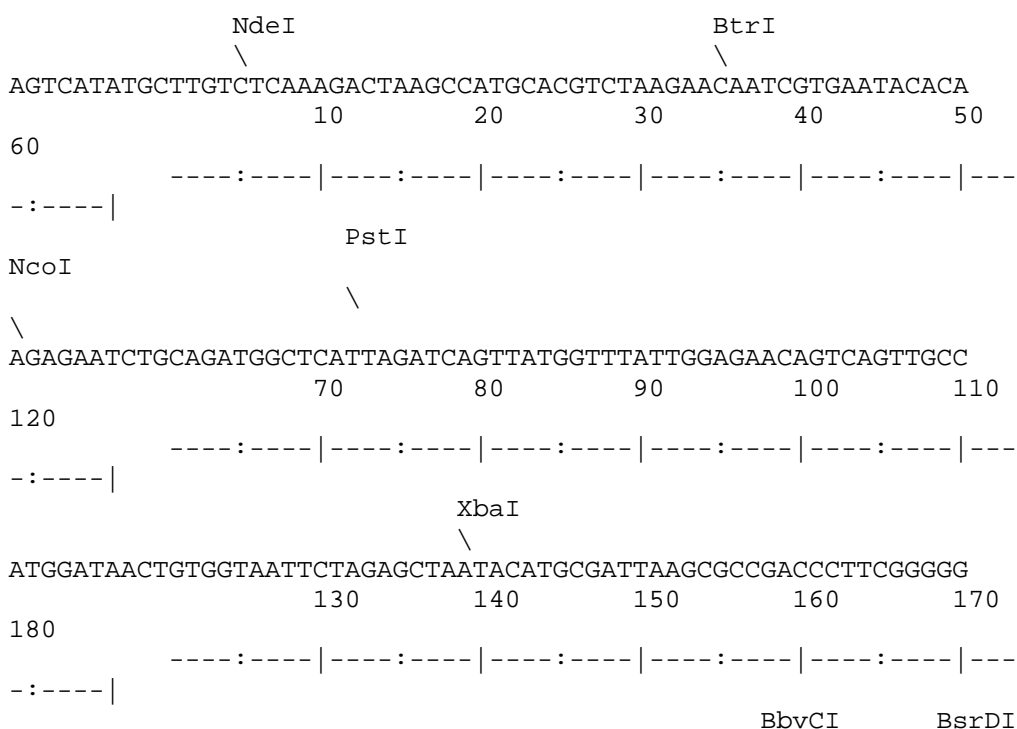
Figure 5.9 Electrophoresis gel showing that ten samples of gut DNA were successfully amplified.

The sequence of the 18S product from NSF4 and NSR581 produced from *H. steineni*, was sequenced to a high quality *via* sub-cloning and Sanger sequencing. This was used as a reference to produce a restriction map of the 566 bp clone. The restriction enzymes in this *H. steineni* map were compared with those of the same sequence in *Corethron inerme* (accession number: AJ535180), which was the only prey item identified in the light microscopy study for which there was a database entry of the same region of the 18S gene. The SEM study was carried out much later and so the data were not available when the restriction enzyme analyses were performed. The restriction enzymes used to cut the *H. steineni* 18S PCR product were chosen on the following basis:

- They all cut at restriction sites comprising a 6 base pair nucleotide recognition sequence
- They did not appear in the *C. inerme* restriction map
- They were commonly available i.e. sold by NEB (the major supplier of restriction enzymes)

Since the sequences of most of the prey items were unknown, three different enzymes were chosen to try and alleviate potential bias in the results.

*H. steineni*_18S



```

Eco31I
\
\
AAGCGTGCTTTTATTAGGAACAAGGCCAGCTCGGTTCCCTCAGCAATGGGGGCCGGTCTCC
190      200      210      220      230
240
----:----|----:----|----:----|----:----|----:----|---
-:----|
      XbaI      PvuI      Eco31I
      \      \      \
GCTGGTGAAC TCTAGATAAC CACGCCGATCGCACGGTCTCGCACCGGCGACGCTCCCTTC
250      260      270      280      290
300
----:----|----:----|----:----|----:----|----:----|---
-:----|
AAAAGTCTGCTCTATCAACTTTTCGATGGTAAGTTATCTGCTTATCATGGTTGTGACGGGT
310      320      330      340      350
360
----:----|----:----|----:----|----:----|----:----|---
-:----|
      BspMII*
      \
AACGGAGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAG
370      380      390      400      410
420
----:----|----:----|----:----|----:----|----:----|---
-:----|
      EcoP15I
      \
GAAGGCAGCAGGCACGCAAATTACCCACTCCCGACACGGGGAGGTAGTGACAAAAAATAA
430      440      450      460      470
480
----:----|----:----|----:----|----:----|----:----|---
-:----|
      StuI      Ksp632I*      AhaIII*
      \      \      \
CGATACAGGCCTCTTTCGGAGGCTCTGTGATCGGAATGAGTACACTTTAAATCCTTTAACG
490      500      510      520      530
540
----:----|----:----|----:----|----:----|----:----|---
-:----|
AGGATCTACTGGAGGGCAAGTCTGGT
550      560
----:----|----:----|----:----|

```

Figure 5.10 18S genetic barcode sequence for *H. steineni*

In an initial trial of this technique, the *H. steineni* 18S sequences were digested and the products purified and reamplified using the same primer set before sub-cloning. It was thought that the cut host DNA would not amplify as it had been cut and therefore each host fragment now only contained one primer site. However, when these PCR products from *H. steineni* were sub-cloned by bacterial transformation, and 12 colonies sequenced in a test run, out of the 72 sequences (12 clones each from PstI, PvuI and StuI digested gut and faeces PCR products), all 72 sequences came back as host (*H. steineni*) DNA barcodes. Hence, simple restriction enzyme digestion and clean-up of the PCR product was insufficient to remove the host DNA. Thus it was decided that it would be necessary to run the restriction enzyme digested PCR product out on a gel and cut out the un-digested prey band and sub-clone this directly. When the restriction enzymes were used to cut the host DNA and bacterial transformations carried out, transformation efficiencies were very poor with only 1-2 colonies per digest and this was not enough for a sequencing screen.

Light microscopy results

Fifteen different genera of phytoplankton were found within the stomach contents of *H. steineni*, of which five were present in all four of the sea cucumber stomachs. Only three items were identified to species level (Table 5.5). These data were based on presence absence and no quantification of each genus was carried out. Identification of the different species can be seen in Appendix 2.1. These data were collected in order to validate the molecular analysis by comparison of prey items found through DNA sequencing.

Phytoplankton species	<i>H. steineni</i>			
	1	2	3	4
<i>Coscinodiscus</i> sp.	X			
<i>Corethron pennatum</i>	X	X	X	X
<i>Fragellariopsis</i> sp.	X	X	X	X
<i>Nitzschia</i> sp.	X	X	X	X
<i>Pseudonitzschia</i> sp.	X	X	X	X
<i>Thalassiosira</i> sp.	X	X	X	X
<i>Cocconeis</i> sp.	X		X	
<i>Pseudogomphonema</i> sp.	X		X	X
<i>Rhizosolenia</i> sp.		X		X
<i>Corethron inermis</i>			X	
<i>Chaetoceros</i> sp.			X	
<i>Odontella</i> sp.			X	
<i>Navicula</i> sp.			X	
<i>Pleurosigma</i> sp.			X	
<i>Melosira</i> sp.				X
<i>Odontella litigiosa</i>				X
Dinoflagellate				X

Table 5.5 Prey items found in the guts of four *H. steineni* under high power (x50 light microscope). Identifications carried out by Dr Jacqueline Stefels, Southern Ocean phytoplankton expert, University of Groningen.

Identification of phytoplankton using the SEM microscope

Four classes, twelve orders, twelve families and fifteen genera of phytoplankton were detected within the stomach contents of *H. steineni* using the SEM (Table 5.6). These data were based on presence/absence, and no quantification of each genus was carried out. In this case, five items were identified to species level. Identification of the different species is presented in Appendix 2.1. These data were collected in addition to the light microscope observations to validate the molecular analysis by comparison of prey items found through DNA sequencing.

Class	Order	Family	Genus
Dictyochophyceae	Dictyochales	Dictyochaceae	<i>Distephanus silicoflagellate</i>
Mediophyceae	Biddulphiales	Biddulphiaceae	<i>Eucampia antarctica</i>
Bacillariophyceae	Corethrales	Corethraceae	<i>Corethron</i> sp
Bacillariophyceae	Thalassiosirales	Thalassiosiraceae	<i>Thalassiosira</i> sp
Bacillariophyceae	Coscinodiscales	Coscinodiscaceae	<i>Coscinodiscus</i> sp
Bacillariophyceae	Achnanthes	Cocconeidaceae	<i>Cocconeis</i> sp
Bacillariophyceae	Naviculales	Pleurosigmaaceae	<i>Pleurosigma</i> sp
Bacillariophyceae	Naviculales	Naviculaceae	<i>Gyrosigma</i> sp
Bacillariophyceae	Naviculales	Naviculaceae	<i>Navicula</i> sp
Bacillariophyceae	Centrales	Chaetocerotaceae	<i>Chaetoceros</i> sp
Bacillariophyceae	Bacillariales	Bacillariaceae	<i>Nitzschia</i> sp
Bacillariophyceae	Rhizosoleniales	Rhizosoleniaceae	<i>Rhizosolenia</i> sp
Bacillariophyceae	Rhizosoleniales	Rhizosoleniaceae	<i>Dactyliosolen girdlebands</i>
Bacillariophyceae	Triceratiales	Triceratiaceae	<i>Odontella</i> sp
Diatomophyceae	Pennales	Bacillariaceae	<i>Fragilariopsis curta</i>
Diatomophyceae	Pennales	Bacillariaceae	<i>Fragilariopsis vanhearackii</i>

Table 5.6 Prey items found in the guts of three *H. steineri* using the scanning electron microscope (SEM). Identifications by Dr Claire Allen, British Antarctic Survey.

Many of the species identified by these two methods overlapped (although there were differences) with the potential reasons for this discussed below. These provided a good basis for the validation of any molecular results.

5.4 Discussion

This study investigated the diet of the filter feeder *H. steineni* using visual observation and tested molecular methods. Discerning the dietary habits of marine invertebrates is important to understanding ecological structure and processes in marine systems. In this study I identified prey items using morphotaxonomic approaches with the aid of experts in the field (Table 5.5 and 5.6 Appendix 2.2). Eighteen different genera of phytoplankton were identified with the majority from the class Bacillariophyceae. Bacillariophyceae grow abundantly in sea waters around the world and are the foundation of the marine food chain (Tahami and Pourgholam 2013). Certain species of prey such as *Eucampia antarctica* and *Distephanus silicoflagellate* were found within the gut contents of *H. steineni* when analysed under the SEM. However they were not found under the light microscope which could be for a number of reasons:

- **Solubility:** *Eucampia antarctica* is easily dissolved and could have therefore dissolved with the ethanol that was used as a preservative during light microscopy rather than it not being present (Allen, C pers comms).
- **Annual variability:** the gut contents of *H. steineni* that were collected for light microscopy were extracted in the austral summer of 2015/16, whereas the gut contents of *H. steineni* collected for SEM were extracted in the austral summer of 2016/17. Different years could mean a difference in phytoplankton bloom diversity and magnitude (Venables, Clarke et al. 2013, Rozema, Venables et al. 2017).

- **Collection timing:** the gut content samples in both years were collected during the austral phytoplankton bloom. However, the composition of the summer phytoplankton blooms change over time and this can affect the species richness of different diatoms. For example, *Chaetoceros* sp (comprising 90% of diatoms found in sea floor sediments) is a pioneer diatom, which rapidly increases in abundance at the start of the summer phytoplankton bloom, outcompeting other diatom species. As the intensity of the bloom declines later into the austral summer then some of the less competitive diatom species may appear (Riebesell 1989).
- **Light and nutrients:** Diatom species such as *Corethron pennatum* and *Rhizosolenia* sp can move up and down in the water column by altering their buoyancy. Therefore they may be present in the shallows for more light and nutrients and then go deeper to avoid predation (Deppeler and Davidson 2017) and thus their presence will vary with sampling depth. *Fragillariopsis* sp is an open ocean diatom but if conditions near the coast are similar (i.e. temperature, light and nutrients), then they may be found nearshore (Beszteri, John et al. 2007) which was evident in the light microscope slides in this study as all four of the *H. steineni* gut contained *Fragillariopsis* sp.
- **Winter fast ice:** *Pseudonitschia* sp are associated with sea ice and therefore the presence of winter fast ice will affect whether they were present within the water column. *Chaetoceros* sp has a preference for a stable water column. If there is a reduction in winter fast ice, there will be more mixing of the water column and therefore less *Chaetoceros* sp and the magnitude of the bloom will be reduced. However, this will also mean the diversity of diatom species may increase (Zielinski and Gersonde 1997, Deppeler and Davidson 2017).
- **Ice scour:** *Cocconeis* sp is a benthic diatom, which normally lives attached to macroalgae. If algae are disturbed by ice scour or rock fall then this diatom could end up in the water column (Totti, Cucchiari et al. 2007).

- **Life span:** The life span of most phytoplankton is still unknown but researchers think two years is an underestimate. Therefore, certain species such as *Cocconeis* sp (benthic diatom) could be present pre and post austral summer phytoplankton bloom (Allen, C pers comms).

Therefore, the morphotaxonomy results almost certainly represent a minimal list of prey items for *H. steineni*. These data highlight the need to carry out seasonal studies on the gut contents of any species, but also the requirement for base-line studies on feeding to investigate the effects of climate change on diet. The climate in Antarctica is predicted to experience increased warming, strengthening winds, acidification, shallowing of mixed layer depths, increased light (and UV), changes in upwelling and nutrient replenishment, declining sea ice, reduced salinity, and the southward migration of ocean fronts (Ducklow, Fraser et al. 2013). These changes are expected to alter the structure and function of phytoplankton communities in the Southern Ocean (Deppeler and Davidson 2017).

Despite all of these problems a total of 18 phytoplankton genera were detected in the guts of *H. steineni*. These ranged over at least 12 orders from benthic, coastal to open ocean. *H. steineni* has a wide catholic diet of phytoplankton.

In conclusion this study aimed to use morphological identification to validate the molecular methods. However, extensive molecular analysis was not possible due to time constraints and the molecular methodology needed significant optimisation. This was not achieved by the time of the thesis submission. The main issue with the methodology was the need to remove the host DNA, which swamped any amplification of gut contents. Some of the previous studies on gut contents have used prey specific primers (Hoogendoorn and Hiempel 2001). However, this was not possible in this study for two reasons:

- **Limited DNA barcoding for prey items:** Genbank only contained the 18S barcode for one of the prey items, *Corethron inerme* which could be due to the difficulty of culturing individual diatom species and also the difficulty of separating species within a diverse water sample.
- **Little prior knowledge of marine invertebrate diets:** Whilst the diet of these species can be guessed at, in general terms (filter feeder, scavenger etc) the knowledge of their diet was not detailed enough to be able to design specific primer sets for the prey.

I successfully amplified DNA for all host species (Appendix 2.2, Figure 5.11) and we successfully amplified the gut contents using the 18S primers. I chose the NSF4 and NSR581 18S primers as they amplified up across all host species and therefore were most likely (out of all the primer sets tested) to amplify across a range of prey items. However, I was unable to successfully sub-clone any prey items within the gut and faeces, probably due to a high quantity of predator DNA present which the initial tests using restriction enzymes failed to remove. I would have expected studies on species such as the cushion star *O. validus* to generate a lot of host DNA as they expel their stomach to digest prey, but even for the sea cucumber *H. steineni*, where the gut contents were easily removed, all the 72 sequences came back as host DNA. Whilst a considerably higher number of sequences could be produced using Next Generation Sequencing of 18S PCR products, due to the issues of host DNA contamination, it was felt that it was necessary to optimise the technique using sub-cloning and Sanger sequencing as a proof of concept and methodological validation first.

After restriction enzyme digestion and cutting out of the undigested band from the gel for sub-cloning then transformation, the results were poor, with very little successful transformation. This was only an initial test, and the protocol clearly needs to be redone with higher quantity of DNA from the prey items, as probably only a small amount of DNA for the prey items was cut from the Gel Green agarose gel.

The PvuI restriction enzyme should cut the host DNA most efficiently compared to the other two enzymes, which both cut at the very ends of the 18S sequence. PvuI cuts the middle of the host 18S sequence at 270 base pairs, producing a bigger distance between the uncut prey and cut host 18S DNA on an agarose gel, thus minimising host contamination when cutting out the gel bands. If given more time to trial further restriction enzyme digests using a larger amount of DNA from the 18S PCR products, this study may have been more successful in obtaining and identifying the sequences of prey items from the gut and faeces of the sea cucumber *H. steineni*. Blankenship and Yayanos (2005) successfully examined the gut contents of marine invertebrates using universal primers and digested PCR products. They found the diets were considerably more diverse than previously thought, which would be the case when compared to the diversity found using the various microscopy studies. However, to date there are only very limited studies using molecular methods to identify prey items from gut and faeces, particularly where the prey items are unknown and prey-specific primers cannot be used.

5.4 Conclusions

The PCR, transformation and restriction enzyme digest method is a potentially powerful technique for expanding the range and diversity of dietary items detected in stomach and faecal contents. However, on this occasion, and with the timescales involved, I was unable to validate the molecular method against morphospecies identification by microscopy. The 18S PCR amplifications have been produced for all the samples collected (gut and faecal contents, five species, two seasons) and will be stored in the -20°C freezer for future investigation.

Chapter 6 – General discussion.
Benthic ecosystems in Antarctica:
biodiversity and energy flow

Chapter 6 General discussion

Benthic ecosystems in Antarctica: biodiversity and energy flow

6.1 Main findings of the study

This thesis has provided the most detailed description and analysis of the biodiversity of a shallow water hard substratum site in Antarctica to date. Furthermore, it is the first to evaluate biodiversity in both summer and winter seasons. The numbers of species present and community structure were in line with expected outcomes and previous results. The data here allowed comparisons with a previous assessment of benthic biodiversity at the same site 17 years previously, which highlighted the effects of increased iceberg scour over that period. The project also made a novel preliminary attempt to start to identify prey of the main components of the Antarctic nearshore benthic ecosystem using traditional microscope and SEM methods combined with a molecular approach. These outcomes are discussed in more detail later in this chapter.

Furthermore, this work was the first to make a comprehensive assessment of the organic mass and carbon content of an Antarctic hard rock community. It measured the quantity of organic carbon across depths, seasons, locations and time, organic carbon that is maintained and used to support metabolic costs and organic carbon that is consumed as prey. This first in depth taxonomic, seasonal, benthic study looking at organic carbon on Antarctic hard rock communities, also adds significant additional pieces to the puzzle of trying to understand the effect climate change will have on marine benthic biodiversity in Antarctica.

Certain aspects of this thesis present new findings across a range of scales, such as:

Heterocucumis steineni, the primary consumer, showed the same factorial increase in oxygen consumption from winter to summer as the secondary consumers *Odontaster validus* and *Ophionotus victoriae*. It was expected that seasonal changes in metabolic rates would vary differently between animals of different trophic levels and that secondary consumers would be less affected by seasonality than primary consumers. The measurements of ash-free dry mass (AFDM) of benthic communities on hard substrata in both summer and winter in the Antarctic, not only quantified the benthic biomass, but showed no significant change from summer to winter. This study also assessed biodiversity across all phyla for organisms <3mm, which provides a very valuable baseline for future assessments of impacts of climate change in this region rather than for one specific animal group, or even a limited range of taxa. This study has provided a much improved perspective of the real biodiversity in the nearshore Sublittoral areas in Antarctica, and especially in the region of the continent that has experienced the most rapid climate change in the southern hemisphere in the last 75 years.

Other aspects of the data in this thesis add to current research such as benthic carbon recycling. From 1998 till this study there was a large reduction in nearshore shallow water biomass. The largest biomass reduction was at 12m with a total average biomass loss of 2026.4g (WM) (2387g in 1998 to 360.6g in 2015). Barnes (2017) reported, Western Antarctic Peninsula (WAP) ice scouring may be recycling 80 000 tonnes of carbon yr⁻¹. Without scouring, such carbon would remain immobilized, and the 2.3% of shelf that is shallow, could be as productive, or possibly even more productive because of macroalgal growth, as all the remaining continental shelf.

Other findings within this thesis are in line and build confidence on previous studies such as the effect of seasonality on benthic community composition. Species richness, density, diversity and biomass on the nearshore shallow water rocky habitats at Rothera Point in this study were not significantly different between seasons. Studies of benthic meiofauna and macrofauna from the soft seabed sites around Rothera Point also found no difference in community composition with different seasons (Vausse, Morley et al. 2018 in press). There was also a lack of temporally significant variation in benthic megafauna at King George Island, Antarctica throughout a 12 month study (Echeverria, Paiva et al. 2005).

Species richness, faunal abundance, diversity and biomass all significantly differed across depths. This was an expected result from visual observations and previous Antarctic benthic studies: Rothera (Barnes and Brockington 2003), King George Island (Echeverria, Paiva et al. 2005), Terra Nova Bay (Gambi, Lorenti et al. 1994), Deception Island (Barnes, Linse et al. 2008) and McMurdo Sound (Dayton, Robilliard et al. 1970) and the results here were in line with previously reported trends.

These points have been discussed in detail within the previous relevant chapters and therefore I shall now consider the main points from the previous chapters in relation to the bigger question raised in the introductory chapter which is “What is the effect of climate change on marine biodiversity of Antarctic hard rock communities: species biomass and energy use”.

6.2 Sea Ice loss

Biomass is related to food availability and for Antarctic benthic marine invertebrates food availability can be seasonal. Food supply is already being affected by climate change, for example the magnitude of the spring phytoplankton bloom is much reduced following winters with the lowest sea ice cover (Venables, Clarke et al. 2013). The annual timing of sea-ice formation and departure is critical to the functioning of polar ecosystems, but in recent decades sea-ice dynamics have been changing.

However, reduced sea ice cover also increases the duration of the phytoplankton bloom. So even though the magnitude of the bloom may be lower, longer duration means a longer period of feeding and therefore potentially more growth and laying down of biomass. Dissecting the differing effects of these factors on benthic communities will not be simple, and will require future studies analysing growth and reproductive effort in a range of species in years of higher intensity, shorter duration phytoplankton blooms and *vice versa* in years of small, longer term blooms. Phytoplankton bloom composition is also likely to have an effect, which will complicate analyses even further.

Barnes and Clarke (1995) showed photoperiod, changes in disturbance by water movement (both mediated by ice) and food concentrations are likely to be important environmental cues for polar suspension feeders. Therefore, less sea ice could mean more feeding by primary consumers and more build-up of biomass. Less winter fast ice brought about by climate change could also allow benthic biofilms to survive into the winter months, supplying food to grazers such as the limpet *Nacella concinna* and the urchin *Sterechinus neumayeri*. Both of these organisms have been shown to cease feeding during the winter months (Fraser, Clarke et al. 2002, Brockington, Clarke et al. 2001). In the past, studies of benthic marine invertebrates have provided evidence of lower biomass in winter compared with summer (Fraser, Clarke et al. 2002). Such expected changes to seasonal dynamics will have knock on effects on the whole system, especially in terms of the timing of events. This is highly likely for reproductive timings, where it is thought that some species key their spawning periods to allow settlement and development of juveniles during periods when phytoplankton productivity is high (e.g. Bowden et al. 2006). Changes in intensity and timing of blooms will have significant implications for these species (see later discussion on seasonality).

Reduced duration of winter fast ice is strongly correlated with increased ice scour and mortality of benthos in the shallows in Antarctica (Barnes and Souster 2011). This study showed a great loss of benthic biomass, mostly from sessile benthic organisms from such disturbance. In my opinion, the loss of biomass due to increased ice scour will outweigh the increase of biomass due to longer duration of the summer phytoplankton bloom, as the growth rates and therefore build-up of biomass in Antarctic marine invertebrates are extremely slow. This effect will last until the recession of coastal ice in the region reaches the point where the majority of glaciers and ice-shelves reach their grounding lines, when the number of icebergs being produced will decrease and thereafter increased productivity will outweigh losses to scour. The timing of these future changes remain very difficult to predict and are essentially unknown.

However, in the coming decades increased ice scour and mortality of benthos will increase the availability of food for scavengers, and therefore there may be a shift in biomass between different trophic levels i.e. loss of biomass from primary consumers and a gain in biomass by secondary and tertiary consumers. As this study shows primary consumers in general have a greater amount of biomass, overall future climate change induced sea ice loss could cause a net loss of Antarctic benthic biomass. In areas protected from scour, dense and diverse biological communities often develop. Seasonal changes in metabolic rates are linked more closely to food availability than temperature change. Given the high diversity and productivity of polar benthic communities (Barnes and Souster 2011), better understanding of their relationship to sea-ice is critical for predicting future polar biodiversity.

6.3 Seasonality

Rocky sub-tidal communities generally contain a wide range of species with highly different life cycles and recruitment strategies (e.g. Bowden et al 2006). Seasonal cues are very likely to be shifting with increases in sea temperature and changes in ice dynamics. With this, reproductive timings could become out of phase with periods when there is optimum food for the juveniles or fall into times when predators are abundant and active.

There is also a remarkable degree of diversity in physiological strategies, cold water adaptations and degree of seasonality within Antarctic marine invertebrates. Seasonality did not affect benthic community composition in this study as most benthic marine invertebrates can withstand significant periods without food (Brockington, Clarke et al. 2001, Fraser, Clarke et al 2002). However, seasonality of food supply drives growth and reproduction, which did in turn have an effect on metabolic rates (Barnes 1995, Grange, Tyler et al. 2004) where oxygen consumption is used to estimate energy use. Metabolic rates in Antarctic marine species appear slowed by the cold rather than compensated (Peck 2016). Metabolism provides energy for all biological functions including the laying down of biomass, which in its simplest terms is the growth of tissues and skeleton.

Energy supply to primary consumers in habitats less than 50m is usually dominated by phytoplankton (Peck 2017). Currently Antarctica experiences extreme seasonality in terms of light and productivity (Clarke 1988). The climate in Antarctica is predicted to experience increased warming, strengthening winds, acidification, shallowing of mixed layer depths, increased light (and UV), changes in upwelling and nutrient replenishment, declining sea ice, reduced salinity and the southward migration of ocean fronts (Ducklow, Fraser et al. 2013). These changes are expected to alter the structure and function of phytoplankton communities in the Southern Ocean (Deppeler and Davidson 2017).

Certain benthic marine invertebrates are efficient at utilizing the low concentration of the microplankton (20 – 200µm) existing in the water column for much of the year (Barnes and Clarke 1995), however, others may require smaller phytoplankton for feeding such as nanoplankton (2 - 20µm). Barnes (2016) found encrusting benthic growth was mainly determined by microalgal bloom duration; each day, nanoplankton exceeded 200µgL⁻¹ it produced ~0.05mm radial growth of some bryozoan species.

6.4 Final thoughts

Over the next few decades there will be a net loss of shallow water benthic biomass in Antarctica due to the near shore shallows being held in early successional stages, with future IPCC climate change scenarios and therefore increased frequency of ice scour. There will be new areas available for colonisation due to ice retreat and ice shelf collapse such as the recent collapse of the Larsen C ice shelf. At depths beyond 50m large new benthic communities will develop, however, in shallow depths these areas will also be subjected to frequent ice scouring preventing large additions to benthic biomass.

The shallows will consist of more mobile benthic organisms and have a lower density of sessile organisms. However, glaciers will eventually reach grounding lines and iceberg production will diminish and the average age of icebergs being ten years means eventually there may be a lower quantity of icebergs. With less ice scour the shallow water benthic communities in Antarctica may become much more highly developed and, as for deeper communities, become dense and diverse. They will then act as a major sink of carbon storage.

6.5 Directions for further research

Although this thesis has been largely successful in its main goals, there are several areas in which more research is required to answer both specific questions such as diet of given species and the big question on the effect of climate change on benthic marine invertebrates over longer temporal scales. It is difficult to make solid conclusions based on small scale, relatively short term studies such as the one reported here about processes, which operate over greater special and decadal scales. The directions for further work are:

- Benthic marine Antarctic invertebrates grow slowly and generally take longer than lower latitude species to reach reproductive maturity. Therefore there is a need for widespread long term benthic biodiversity monitoring to be able to assess future change.
- Multi year benthic biodiversity surveys are needed to be able to draw solid conclusions on temporal scales. This study would have been enhanced by a biodiversity and biomass study carried out in additional years to increase the ability to evaluate inter annual variation. However, the current project has set up a strong baseline from which to build on and to assess future changes.
- There are long term data on factors affecting the benthos such as fast ice duration, phytoplankton bloom and light from the RaTS program. Multiple year's benthic biodiversity and biomass data would make robust testing of seasonal differences much more reliable and assessments of the effects of seasonal changes in environmental variables possible.
- Improved taxonomic identification would enhance the power of the analyses in studies of the type conducted here. For example polychaetes can account for more than 70% of benthic fauna at a given site, yet their taxonomic identifications are poorly known and the majority of identifications here were to family level only.

- Fine tuning of dietary analysis using molecular methods for both gut and faecal contents to better understand the diet of benthic marine invertebrates of hard substrata in the Antarctic, would allow energy flows in benthic food webs to be quantified. This would give significant improvement in the understanding of these communities living in some of the coldest, most seasonal and most physically disturbed marine environments on Earth.

Overall this thesis has presented the most detailed and comprehensive analysis of the biodiversity of an Antarctic shallow water rocky benthic site. It has produced novel research in a range of areas from species abundance and biomass measures through seasonal ecology and physiology to molecular approaches to analysing diet. It has further identified several areas for future research, but possibly most significantly, it has provided the best platform for future monitoring and targeted research projects for assessing the impacts of altered environments in a region where climate has changed faster than anywhere else in the southern hemisphere over the last 75 years.

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Appendix 1

Appendix 1 Supporting material for Chapter 2

1.1 Biodiversity and biomass table

Taxa	Mean density m ⁻²			Mean biomass (AFDM) g m ⁻²		
	6m	12m	20m	6m	12m	20m
<i>Nacella concinna</i>	296.9	199.0	40.2	7.92	10.88	3.14
<i>Lothia sp</i>	0.0	4.2	29.3	0.00	0.01	0.12
<i>Eatoniella caliginosa</i>	0.0	29.0	107.2	0.00	0.02	0.08
<i>Eatoniella cf. glacialis</i>	0.0	1.0	15.4	0.00	0.00	0.00
<i>Eatoniella sp</i>	0.0	0.4	2.3	0.00	0.00	0.00
<i>Margarella antarctica</i>	0.0	4.0	25.3	0.00	0.02	0.14
<i>Onoba grisea</i>	0.0	3.4	11.8	0.00	0.00	0.00
<i>Onoba cf. turqueti</i>	0.9	13.0	1.3	0.00	0.00	0.00
<i>Laevilitorina antarctica</i>	0.0	3.0	0.0	0.00	0.00	0.00
<i>Naticidae (family)</i>	0.0	1.4	0.2	0.00	0.00	0.00
<i>Toledonia cf. globosa</i>	0.0	0.0	0.0	0.00	0.00	0.00
<i>Charcotia granulosa</i>	0.0	0.4	1.1	0.00	0.00	0.01
<i>Pseudotrionia quadrangularis</i>	0.0	0.2	0.0	0.00	0.00	0.00
<i>Cuthona cf. modesta</i>	0.0	0.0	0.4	0.00	0.00	0.00
<i>Philobrya wandelensis</i>	0.0	21.8	67.4	0.00	0.02	0.07
<i>Philobrya sublaevis</i>	0.0	0.4	6.5	0.00	0.00	0.03
<i>Adacnarca nitens</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Cyamiomactra laminifera</i>	1.1	9.6	6.3	0.00	0.04	0.02
<i>Limatula ovalis</i>	0.0	0.2	2.3	0.00	0.00	0.02
<i>Aequiyoldia eightsi</i>	0.0	0.4	0.2	0.00	0.00	0.00
<i>Altenaeum charcoti</i>	0.0	1.8	12.6	0.00	0.00	0.01
<i>Melanella sp</i>	0.0	0.0	0.4	0.00	0.00	0.00
<i>Tonicina zschau</i>	3.1	9.0	26.9	0.01	0.04	0.19
<i>Hemiarthrum setulosum</i>	0.0	0.0	0.8	0.00	0.00	0.00
<i>Leptochiton kerguelensis</i>	0.0	0.4	0.6	0.00	0.00	0.00
<i>Liothyrella uva antarctica</i>	0.0	0.4	2.9	0.00	0.00	0.34
<i>Odontaster validus</i>	8.2	27.6	65.7	3.77	4.16	6.76
<i>Odontaster roseus</i>	0.2	0.0	0.4	0.04	0.00	0.10
<i>Odontaster sp</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Cryptasterias turqueti</i>	0.0	1.8	2.3	0.00	9.65	10.35
<i>Diplasterias brucei</i>	0.0	4.4	18.7	0.00	0.13	0.84
<i>Cuenotaster involutus</i>	0.0	0.0	0.4	0.00	0.00	4.88
<i>Pisaster ochraceus</i>	0.0	0.0	4.0	0.00	0.00	0.00
<i>Sterechinus neumayeri</i>	17.3	13.8	41.9	6.99	3.96	6.04
<i>Ophionotus victoriae</i>	0.0	6.0	33.7	0.00	1.41	6.58
<i>Ophiura crassa</i>	0.0	0.0	6.5	0.00	0.00	0.10
<i>Ophiopsammus maculata</i>	0.0	0.0	2.7	0.00	0.00	0.00
<i>Heterocucumis steineni</i>	0.0	1.4	8.0	0.00	4.92	36.33
<i>Echinopsolus charcoti</i>	0.0	2.8	17.3	0.00	1.46	8.83
<i>Echinopsolus acanthocola</i>	0.0	0.0	0.2	0.00	0.00	0.62
<i>Cucumaria sp</i>	0.0	0.8	28.0	0.00	0.39	3.79

<i>Lysasterias sp</i>	0.0	0.0	0.9	0.00	0.00	0.01
<i>Pseudostichopus peripitus</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Prostebbingia brevi/longicornis</i>	39.6	21.4	1.7	0.37	0.19	0.01
<i>Schraderia gracilis</i>	4.9	8.8	11.4	0.01	0.03	0.02
<i>Prostebbingia gracilis</i>	0.7	9.6	0.2	0.00	0.02	0.00
<i>Eurymera monticulosa</i>	64.4	1.4	0.0	0.43	0.01	0.00
<i>Paramoera sp.</i>	0.2	0.0	0.0	0.00	0.00	0.00
<i>Gondogeneia sp.</i>	24.9	4.4	1.1	0.07	0.01	0.00
<i>Rhachotropis antarcticus</i>	0.0	0.2	0.0	0.00	0.00	0.00
<i>Phoxocephalinae</i>	0.0	1.2	0.2	0.00	0.00	0.00
<i>Cheirimedon femoratus</i>	0.0	0.2	0.0	0.00	0.00	0.00
<i>Heterophoxus videns</i>	0.0	0.2	0.6	0.00	0.00	0.00
<i>Parhalimedon turqueti</i>	0.2	3.6	7.4	0.00	0.00	0.01
<i>Methalimedon nordenskjoldi</i>	0.0	0.0	1.5	0.00	0.00	0.00
<i>Oradarea sp.</i>	2.4	0.6	0.4	0.01	0.00	0.00
<i>Oradarea cf. walkeri</i>	0.0	1.0	0.4	0.00	0.00	0.00
<i>Monoculodes scabriculosus</i>	0.0	0.4	0.6	0.00	0.00	0.00
<i>Liljeborgia sp.</i>	0.0	0.4	1.1	0.00	0.00	0.00
<i>Paraceradocus miersi</i>	0.0	1.4	2.9	0.00	0.08	0.33
<i>Cumacea</i>	0.0	0.8	1.5	0.00	0.00	0.00
<i>Iathrippa cf. sarsi</i>	1.6	6.4	1.3	0.00	0.02	0.00
<i>Munna antarctica</i>	2.0	8.8	12.8	0.00	0.01	0.01
<i>Natatolana sp</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Janiridae</i>	0.0	0.2	0.2	0.00	0.00	0.00
<i>Gnathiidae</i>	0.0	0.2	0.0	0.00	0.00	0.00
<i>Cymodocella tubicauda</i>	0.7	18.8	51.8	0.00	0.07	0.24
<i>Austropallene sp</i>	0.0	0.4	8.8	0.00	0.01	0.18
<i>Achelia sp</i>	0.0	5.2	21.1	0.00	0.01	0.06
<i>Pantopipetta sp</i>	0.0	0.2	2.3	0.00	0.00	0.00
<i>Pentanymphe antarcticum</i>	0.0	0.2	4.4	0.00	0.00	0.03
<i>Australis sp</i>	0.0	0.0	0.8	0.00	0.00	0.00
<i>Pycnogonum sp</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Dendrilla antarctica</i>	0.0	0.2	1.1	0.00	0.16	0.06
<i>Haliclona sp1</i>	0.0	0.2	1.9	0.00	0.12	0.17
<i>Haliclona sp2</i>	0.0	0.0	1.1	0.00	0.00	0.51
<i>Pachychalina sp1</i>	0.0	0.2	0.4	0.00	0.01	0.51
<i>Iophon hesperidesi rios</i>	0.0	0.0	0.8	0.00	0.00	0.04
<i>Megaciella sp</i>	0.0	0.0	0.8	0.00	0.00	0.22
<i>Myxilla ectyomyxilla</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Sphaerotylus antarcticus</i>	0.0	0.0	0.4	0.00	0.00	1.20
<i>Suberites topsenti</i>	0.0	0.0	0.4	0.00	0.00	0.08
<i>Protosuberites sp1</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Desmarestia menziesii</i>	0.0	0.4	0.4	0.00	0.08	0.38
<i>Adenocystis uticularis</i>	1.1	0.0	0.2	0.12	0.00	0.00
<i>Kallymenia antarctica</i>	0.0	0.0	0.6	0.00	0.00	0.00

<i>Rhodymenia subantarctica</i>	0.0	0.0	1.3	0.00	0.00	0.00
<i>Palmaria decipens</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Hyenocladopsis crustigena</i>	0.0	0.0	0.0	0.00	0.00	0.00
<i>Plocamium sp</i>	0.0	0.0	0.4	0.00	0.00	0.00
<i>Mixed Algae</i>	0.2	0.4	0.7	0.10	0.16	6.41
<i>Cnemidocarpa verrucosa</i>	0.0	3.0	24.6	0.00	0.11	8.66
ASC01	0.0	0.2	0.6	0.00	0.00	0.02
ASC02	0.0	0.2	2.7	0.00	0.00	0.01
ASC03	0.0	0.0	0.4	0.00	0.00	0.00
<i>Alcyonium antarcticum</i>	0.0	0.0	1.7	0.00	0.00	1.64
CNI01	0.0	0.0	0.4	0.00	0.00	0.17
CNI02	0.0	0.0	0.2	0.00	0.00	0.00
CNI03	0.0	13.4	6.1	0.00	0.02	0.01
<i>Parborlasia corrugatus</i>	8.4	30.4	10.9	0.09	0.50	2.86
<i>Cerebratulus cf longifissus</i>	0.7	0.4	2.7	0.09	0.11	0.29
<i>Baseodiscus cf antarcticus</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Capitellidae</i>	2.0	2.4	4.0	0.00	0.01	0.01
<i>Hesionidae</i>	0.0	0.6	4.4	0.00	0.00	0.01
<i>Nereididae 1</i>	1.3	1.6	2.3	0.01	0.00	0.04
<i>Nereididae 2</i>	2.9	5.4	6.7	0.04	0.04	0.05
<i>Opheliidae 1</i>	0.0	0.2	1.5	0.00	0.00	0.00
<i>Orbiniidae 1</i>	0.4	8.8	5.1	0.00	0.05	0.02
<i>Orbiniidae 2</i>	0.0	1.0	0.4	0.00	0.01	0.00
<i>Polynoidae 1</i>	0.2	17.4	55.8	0.03	0.88	1.92
<i>Polynoidae 2</i>	0.0	0.8	12.8	0.00	0.01	0.27
<i>Sabellidae 1</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Syllidae 1</i>	0.0	20.4	23.2	0.00	0.03	0.03
<i>Syllidae 2</i>	1.1	3.6	0.0	0.00	0.00	0.00
<i>Thelepus cincinnatus</i>	4.2	13.0	53.5	0.03	3.28	21.73
<i>Terebellidae 1</i>	0.0	0.6	2.1	0.00	0.01	0.02
<i>Terebellidae 2</i>	0.0	1.2	0.2	0.00	0.04	0.00
<i>Terebellidae 4</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Aglaophamus trissophyllus</i>	0.2	1.2	2.1	0.16	0.32	0.62
<i>Protolaeospira stalagmia</i>	0.2	23.8	166.1	0.00	0.03	0.23
<i>Romanchella perrieri</i>	0.0	0.8	10.5	0.00	0.00	0.01
<i>Acotylea (sub order)</i>	0.2	0.2	0.0	0.00	0.00	0.00
<i>Prosthiostomidae (family)</i>	0.0	0.0	0.2	0.00	0.00	0.00
<i>Stylochooides albus</i>	0.0	0.4	0.8	0.00	0.00	0.00
<i>Sipuncula</i>	0.0	2.8	2.1	0.00	0.02	0.03
<i>Nematoda</i>	0.0	0.0	1.9	0.00	0.00	0.00

List of taxa found in the samples and their abundance (number of individuals m⁻²) and biomass (mean AFDM g m⁻²) except bryozoans.

Appendix 1 Supporting material for Chapter 2

1.2 Photo identifications of species found within the biodiversity survey Pictures to accompany IDs from Table 2.2 Chapter 2 within the thesis

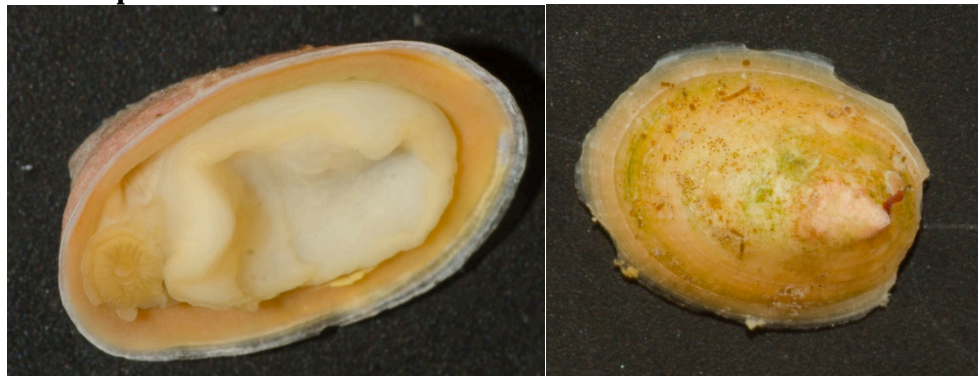
Phylum Mollusca

Class Gastropoda

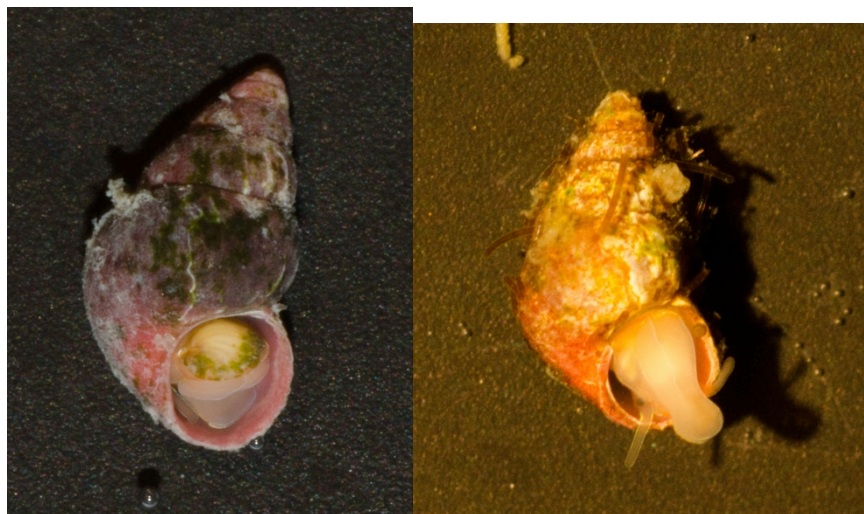
Nacella concinna (Strebel, 1908)



Iothia sp



Eatoniella caliginosa (E.A.Smith, 1975)



***Eatoniella cf. glacialis* (E.A. Smith, 1907)**



***Margarella antarctica* (Lamy, 1905)**



***Onoba grisea* (Martens, 1885)**



Laevitorina antarctica (E.A. Smith, 1902)



Trophon cf. minutus Melvill & Standen, 1907



Melanella sp



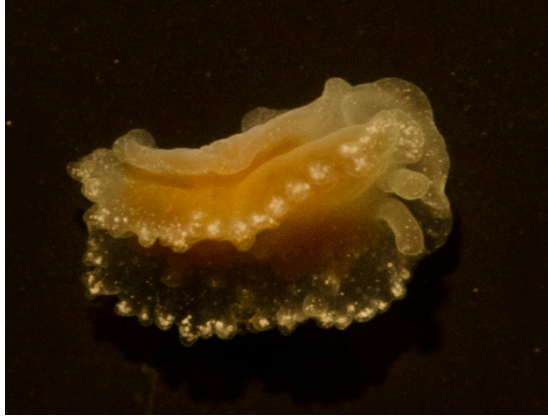
Charcotia granulosa Vayssière, 1906



Cuthona cf. modesta (Eliot, 1907)



***Pseudotriltonia quadrangularis* Thiele, 1912**

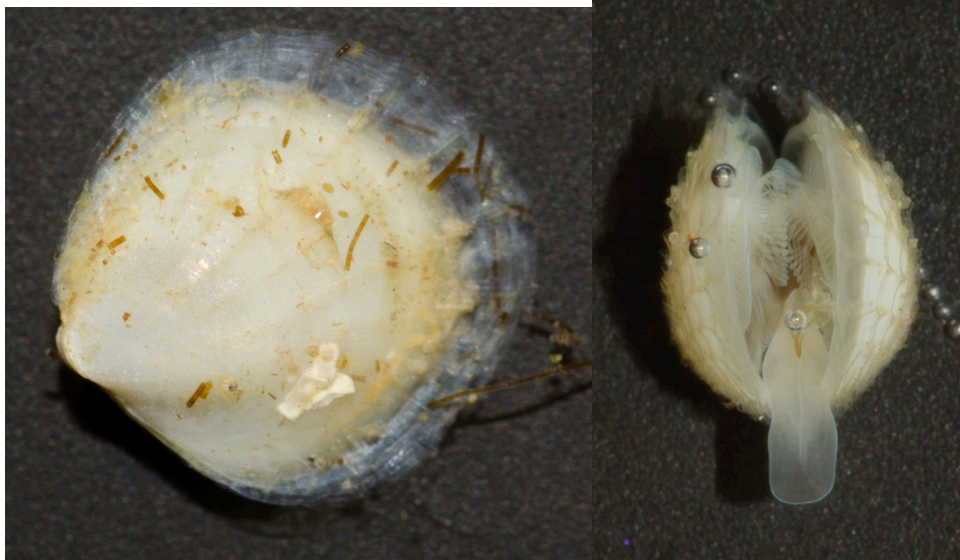


Class Bivalvia

***Philobrya wandelensis* Lamy, 1906**



***Philobrya sublaevis* Pelseneer, 1903**



***Adacnarca nitens* Pelseneer, 1903**



***Cyamiomactra laminifera* (Lamy, 1906)**



***Limatula ovalis* (Thiele, 1912)**



***Aequiyoldia eightsi* (Jay, 1839)**



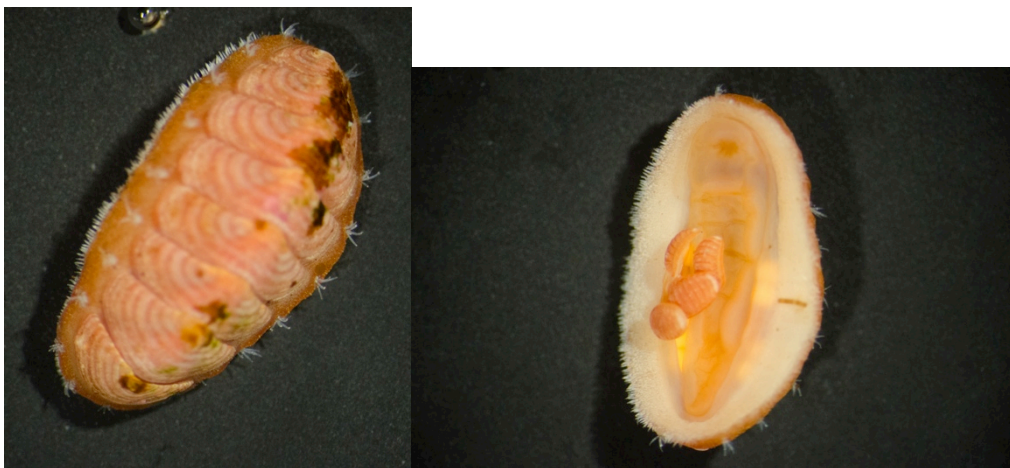
***Altenaeum charcotti* (Lamy, 1906) “ formerly known as *Mysella charcoti* (Lamy, 1906)”**



Class Polyplacophora
***Tonicina zschaui* (Pfeffer, 1886)**



***Hemiarthrum setulosum* Carpenter in Dall, 1876**



***Leptochiton kerguelensis* Haddon, 1886**



**Phylum Brachiopoda
Class Rhynchonellata
Liothyrella uva (Broderip, 1833)**



Phylum Echinodermata
Class Asteroidea
Odontaster validus Koehler, 1906



Odontaster roseus Janosik & Halanych, 2010



Cryptasterias turqueti (Koehler, 1906)



Diplasterias brucei (Koehler, 1907)



Cuenotaster involutus (Koehler, 1912)



Pisaster ochraceus (Brandt, 1835)



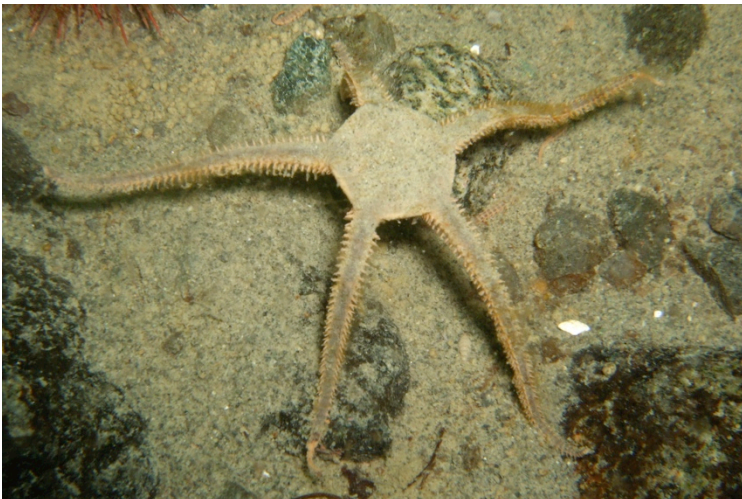
Class Echinoidea

***Sterechinus neumayeri* (Meissner, 1900)**

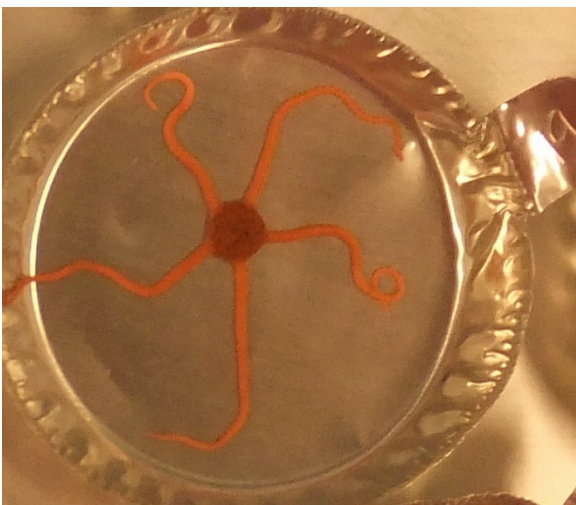


Class Ophiuroidea

***Ophionotus victoriae* Bell, 1902**



***Ophiura crassa* Mortensen, 1936**



***Ophiopsammus maculata* (Verrill, 1869)**



Class Holothuroidea
***Heterocucumis steineni* (Ludwig, 1898)**



***Echinopsolus charcoti* (Vaney, 1906)**



Echinopsolus acanthocola Gutt, 1990 (no image)

Cucumaria sp



Pseudostichopus peripatus (Sluiter, 1901)



Phylum Arthropoda

Class Malacostraca

Order Amphipoda

Prostebbingia brevi/longicornis (Chevreux, 1906)



Schraderia gracilis Pfeffer, 1888



Prostebbingia gracilis (Chevreux, 1912)



Eurymera monticulosa Pfeffer, 1888



Paramoera sp



Gondogeneia sp



Rhachotropis antarctica K.H Barnard, 1932



Phoxocephalinae sp



***Heterophoxus videns* K. H. Barnard 1930**



***Cheirimedon femoratus* (Pfeffer, 1888)**



***Parhalimедon turqueti* Chevreux, 1906**



***Methalimедon nordenskjöldi* Schellenberg, 1931**



***Oraderea* sp**



Monoculodes scabriculosus K.H.Barnard, 1932



Liljeborgia sp



***Paraceradocus miersi* (Pfeffer, 1888)**



Order Isopoda
***Iathrippa cf. sarsi* (Pfeffer, 1887)**



***Munna antarctica* (Pfeffer, 1887)**



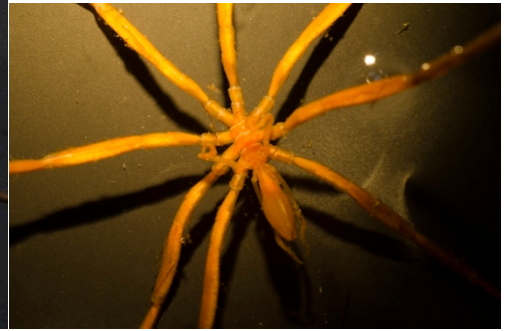
***Cymodocella tubicauda* Pfeffer, 1887**



Class Pycnogonida
***Austropallene* sp**



Achelia sp



Pantopipetta sp



Pentanymphe antarcticum Hodgson 1904



Australis sp



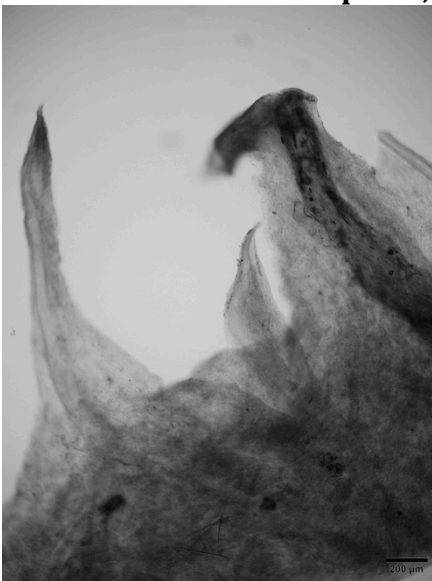
***Pycnogonum* sp**



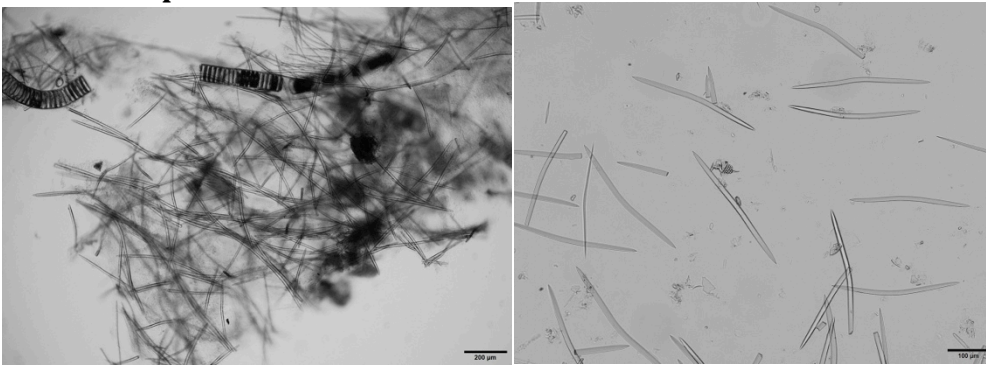
Phylum Porifera (Skeleton and spicule images used for species identification supplied by Dr Claire Goodwin)

Class Demospongiae

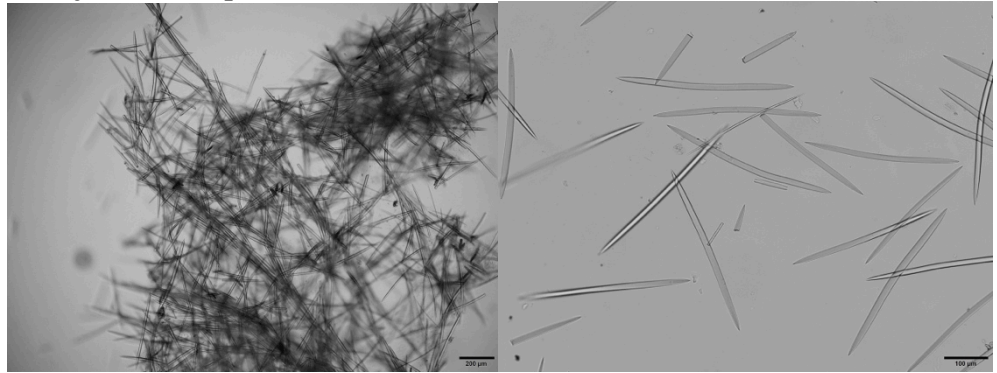
***Dendrilla antarctica* Topsent, 1905**



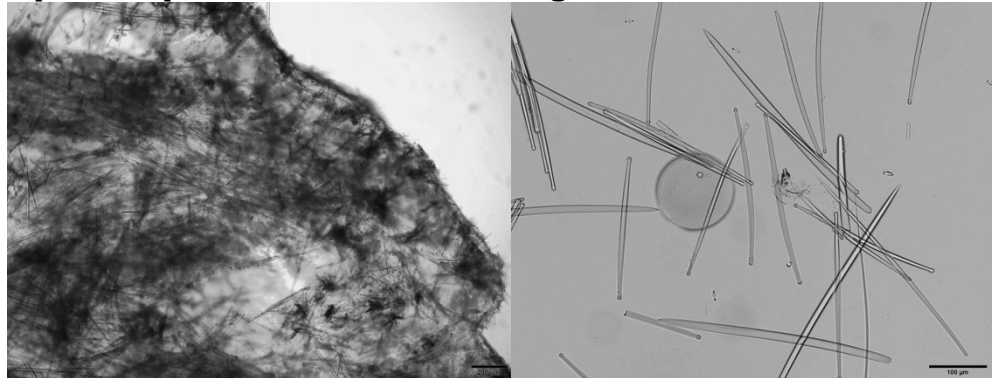
***Haliclona* sp**



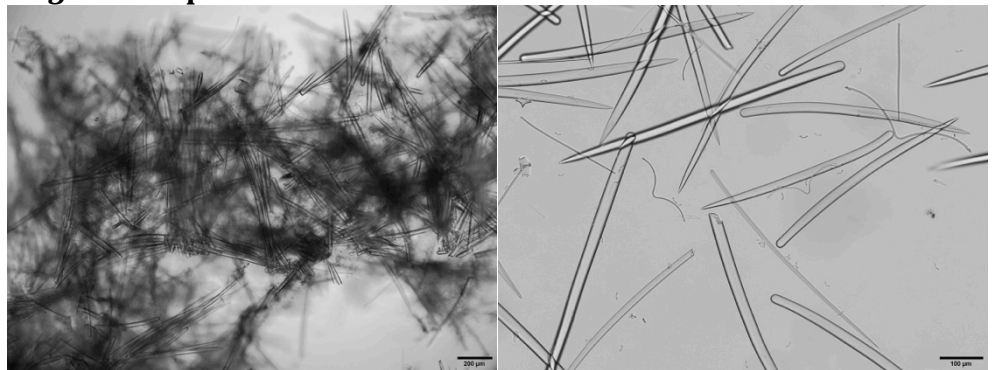
***Pachychalina* sp**



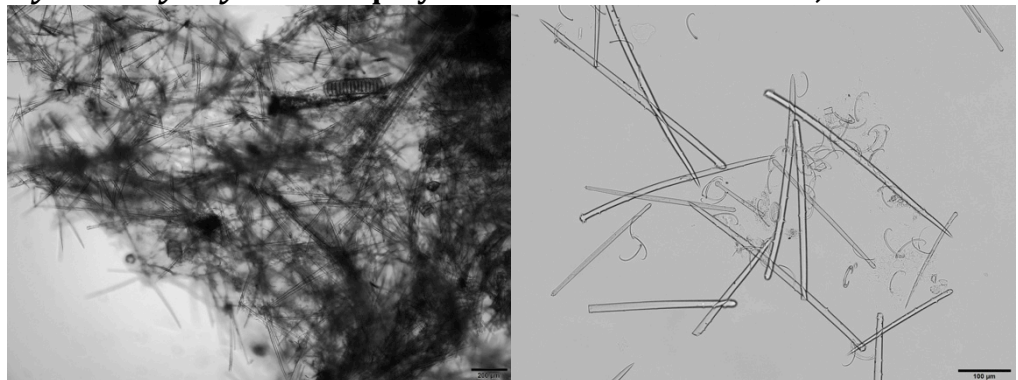
***Iophon hesperidesi* rios Cristobo & Urgorri, 2004**



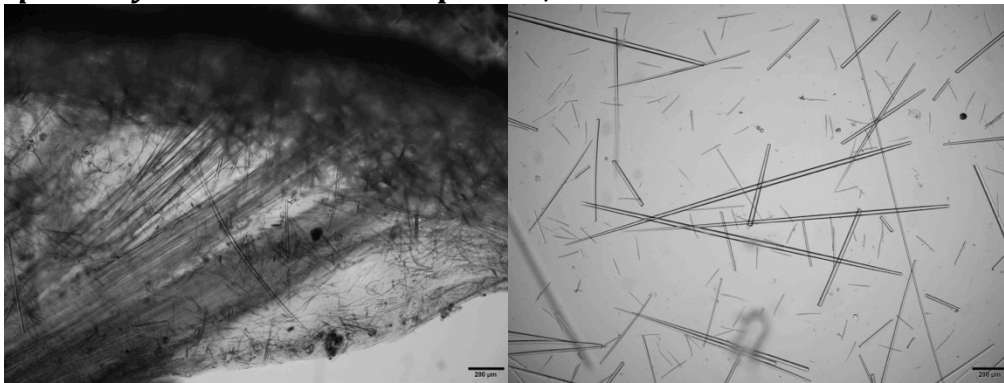
***Megaciella* sp**



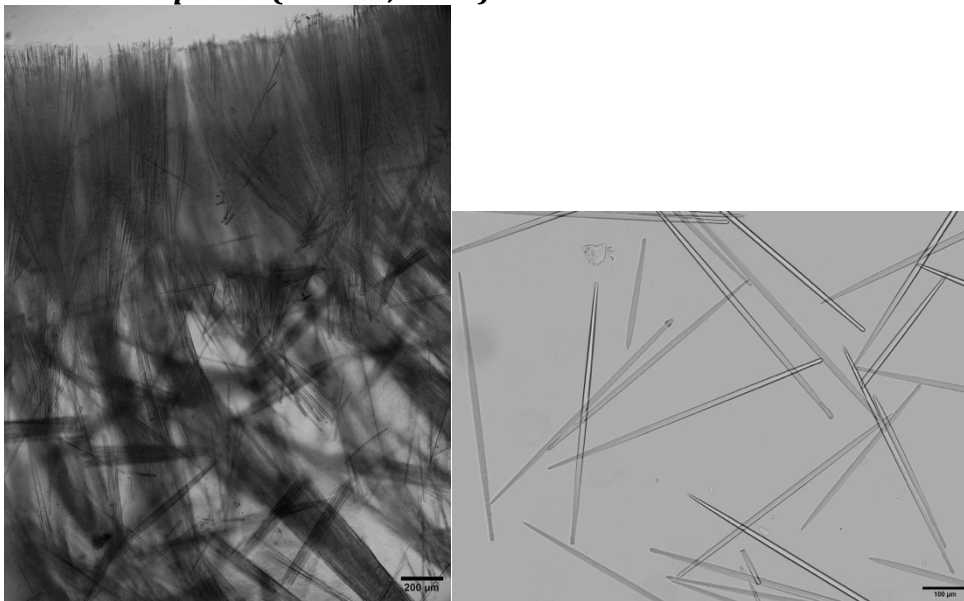
***Myxilla ectyomyxilla* Desqueyroux-Faúndez & van Soest, 1996**



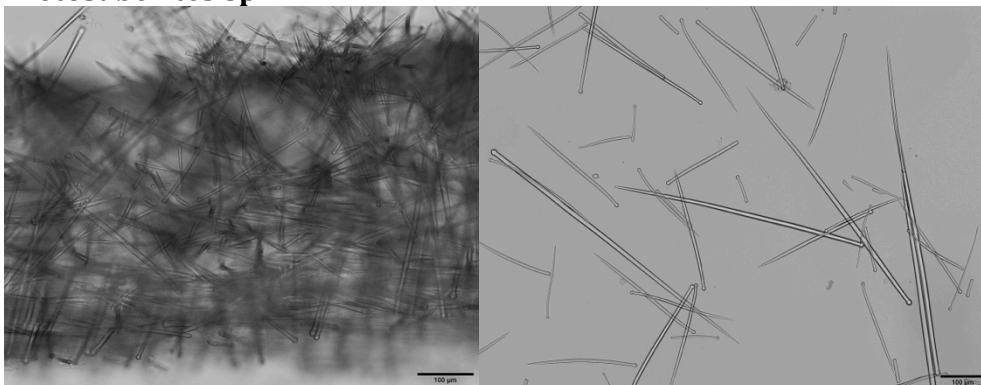
***Sphaerotylus antarcticus* Kirkpatrick, 1907**



***Suberites topsenti* (Burton, 1929)**



***Protosuberites* sp**



Phylum Ochrophyta
Class Phaeophyceae
***Desmarestia menziesii* J. Agardh**



***Adenocystis utricularis* (Bory de Saint - Vincent) Skottsberg, 1907**



Phylum Rhodophyta
Class Florideophyceae
***Kallymenia antarctica* Hariot, 1907**



***Rhodymenia subantarctica* Ricker, 1987**



***Hymenocladopsis crustigena* Moe, 1986**
(Unable to provide image as specimen damaged)

***Palmeria decipiens* Ricker, 1987**



Phylum Chordata
Class Ascideacea
***Cnemidocarpa verrucosa* (Lesson, 1830)**



Phylum Cnidaria
Class Anthazoa
***Alcyonium antarcticum* Wright & Struder, 1889**



Phylum Nemertea
Class Anopla
***Parbolasia corrugatus* (McIntosh, 1876)**



***Heteronemertes cf longifissus* (Hubrecht, 1887)**



***Baseodiscus cf antarcticus* (Bayliss, 1915)**



Phylum Annelida

Class Polychaeta

***Thelepus cincinnatus* (Fabricius, 1780)**



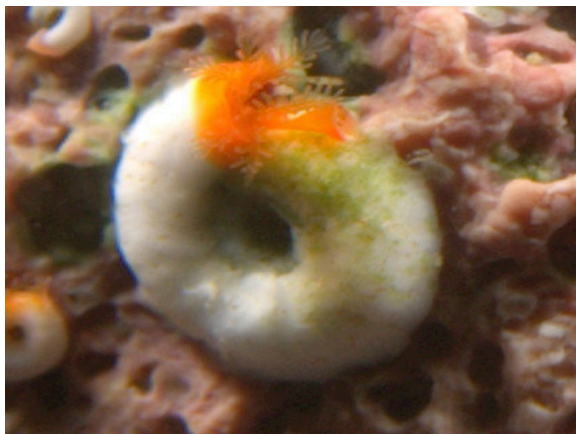
Aglaophamus trissophyllus (Grube, 1877)



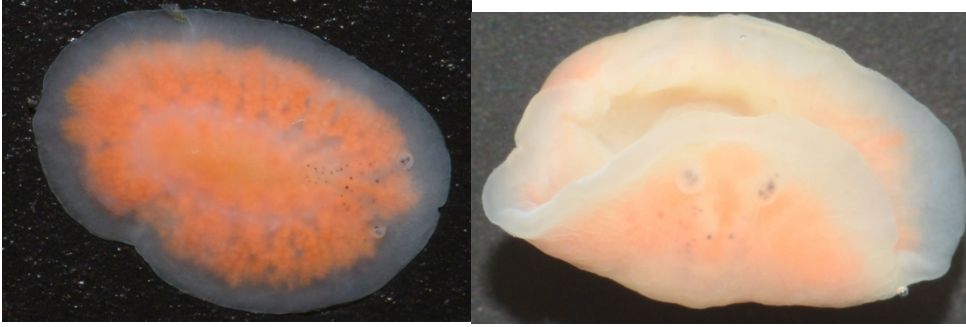
Protolaeospira stalagmia Knight-Jones & Walker, 1972



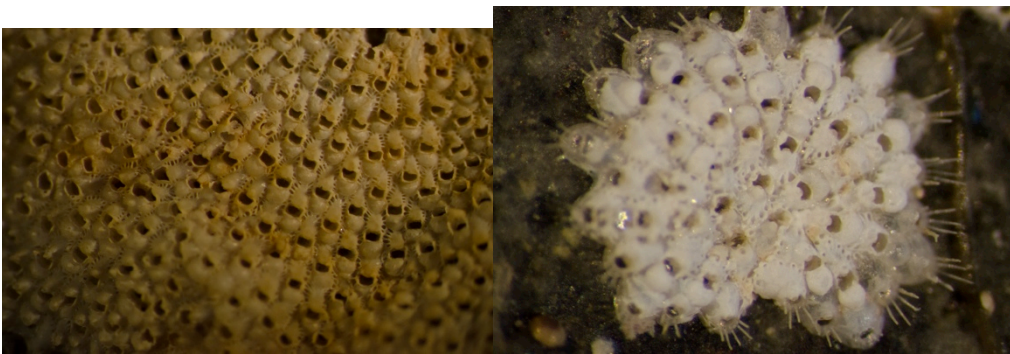
Paralaeospira levinseni (Caullery & Mesnil, 1897)



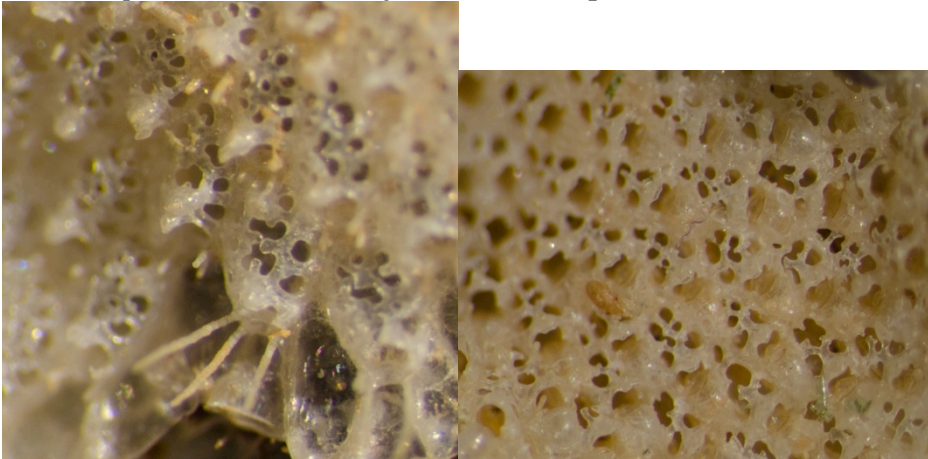
Phylum Platyhelminthes
Class Rhabditophora
***Stylochoides albus* (Hallez, 1905)**



Phylum Bryozoa
Class Gymnolaemata
***Aimulosia antarctica* (Powell, 1967)**



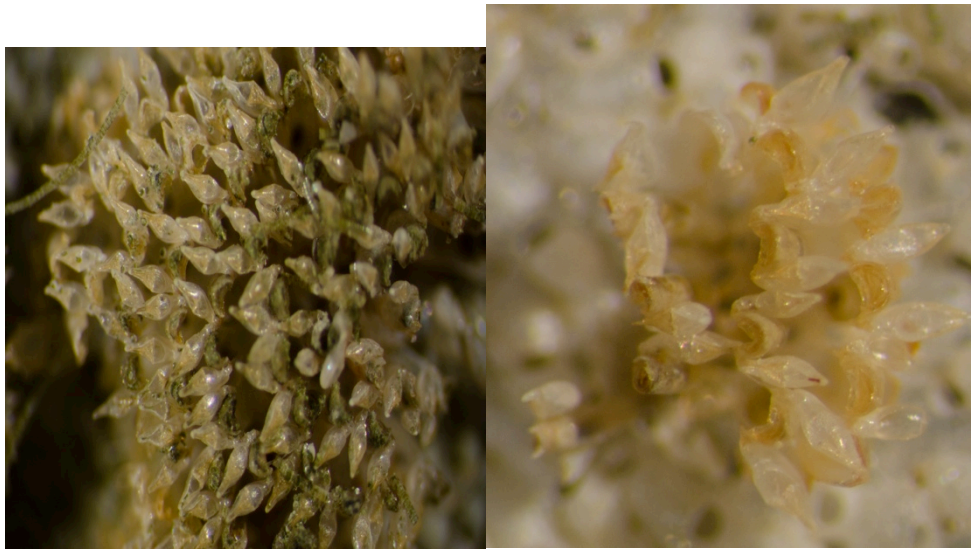
***Arachnopusia inchoata* Hayward & Thorpe, 1988**



***Beania costata* (Busk, 1876)**



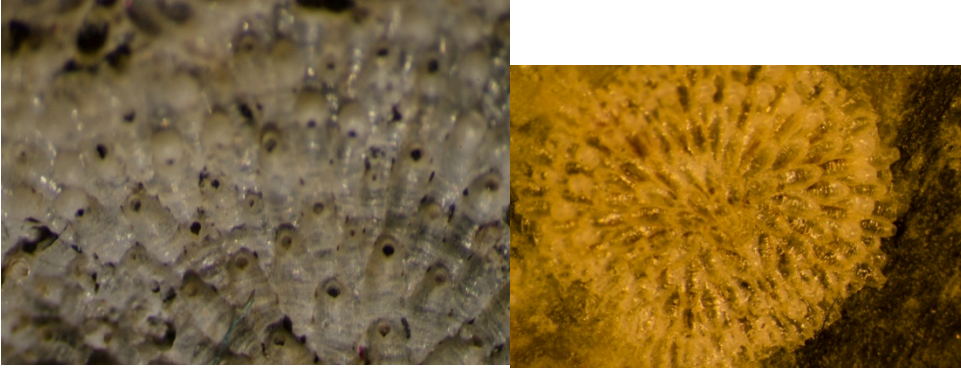
***Beania erecta* Waters 1904**



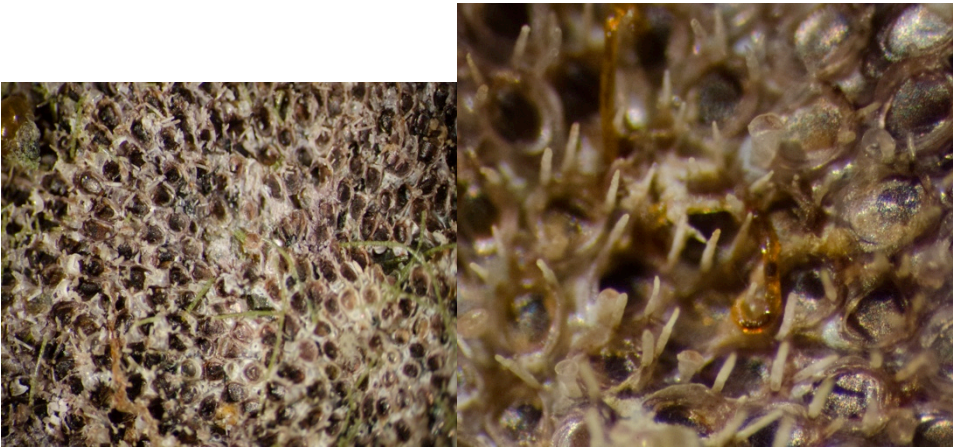
***Camptoplities bicornis* (Busk, 1884)**



***Celleporella antarctica* Moyano & Gordon, 1980**



***Chaperiopsis protecta* (Waters, 1904)**



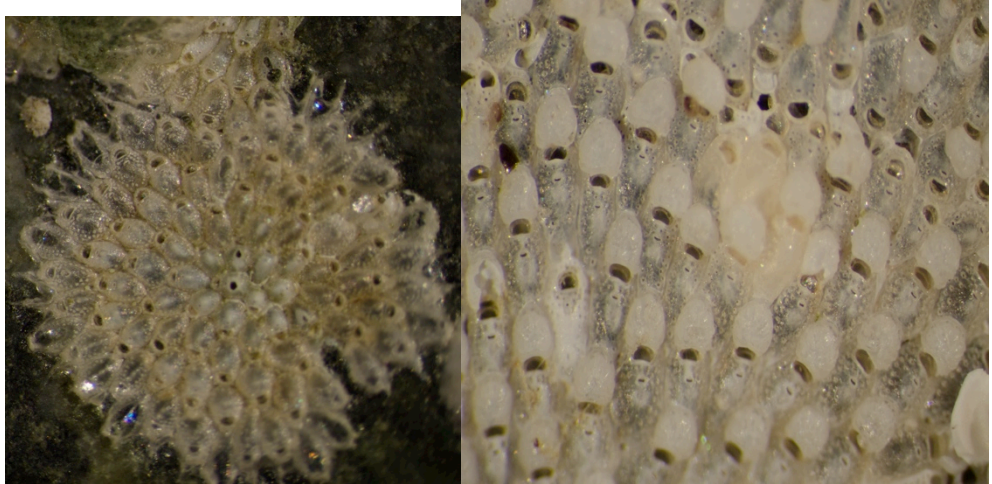
***Ellisina antarctica* Hastings, 1945**



***Escharoides tridens* (Calvet, 1909)**



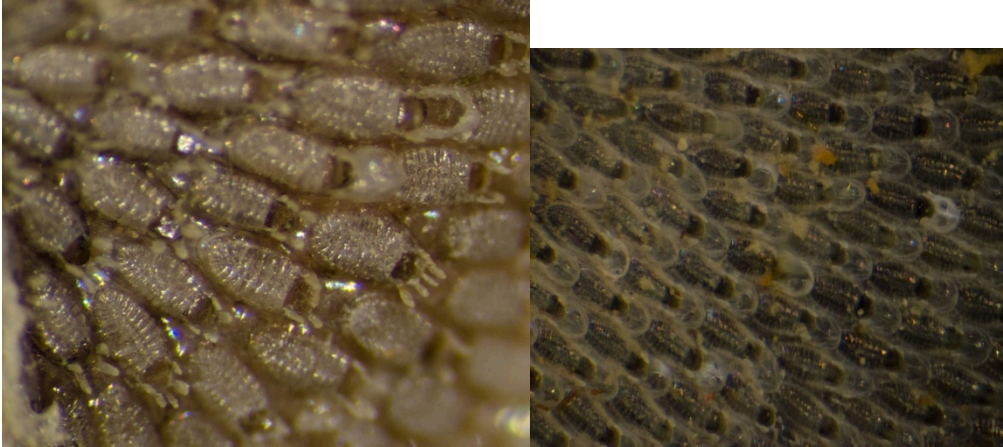
***Fenestrulina rugula* Hayward & Ryland, 1990**



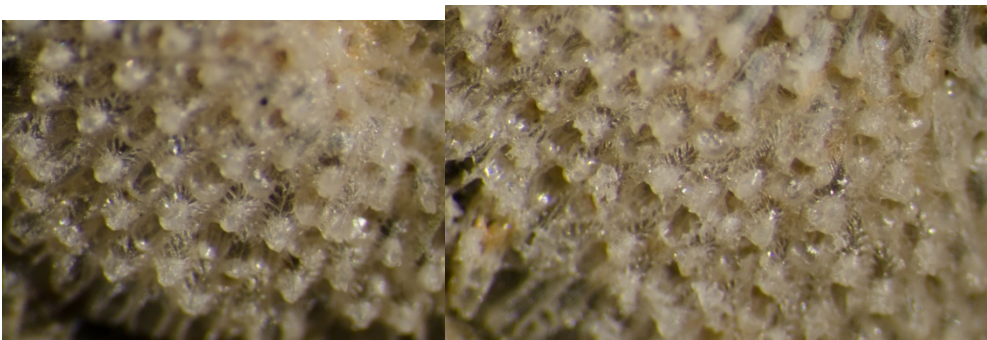
***Figularia discors* Hayward & Taylor, 1984**



***Filaguria spatulata* (Calvet, 1909)**



***Hippadenella inerma* (Calvet, 1909)**



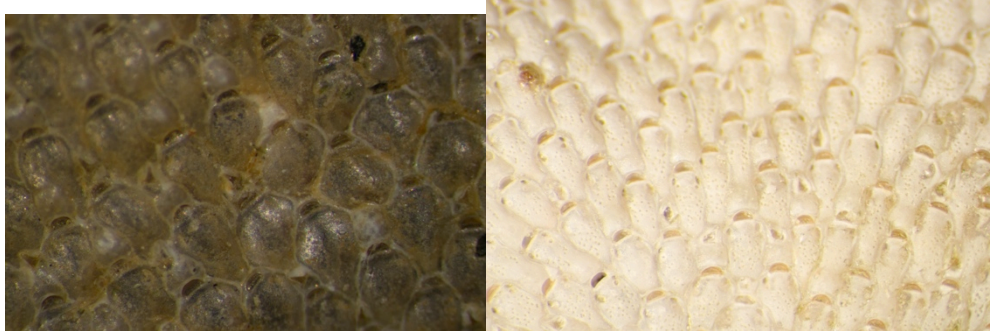
***Himantozoum antarcticum* (Calvet, 1909)**



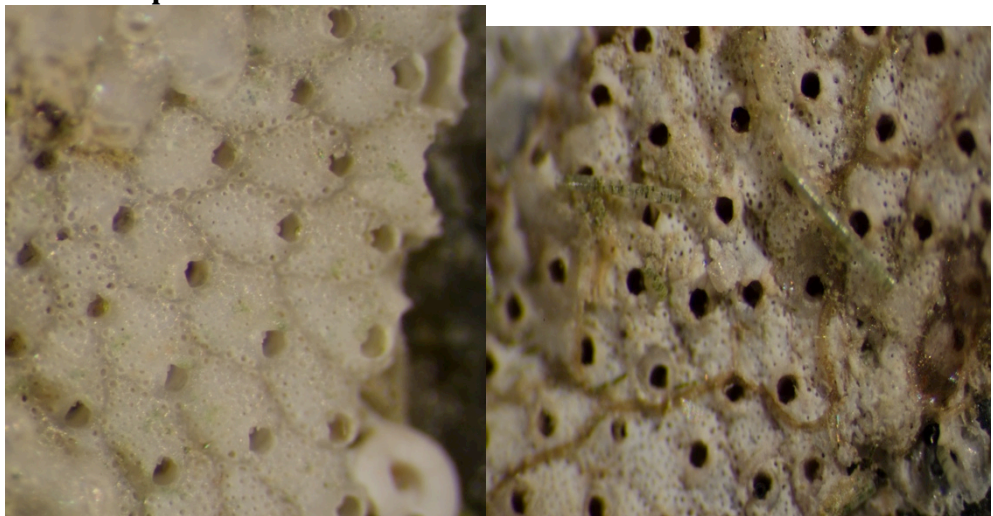
***Kymella polaris* (Waters, 1904)**



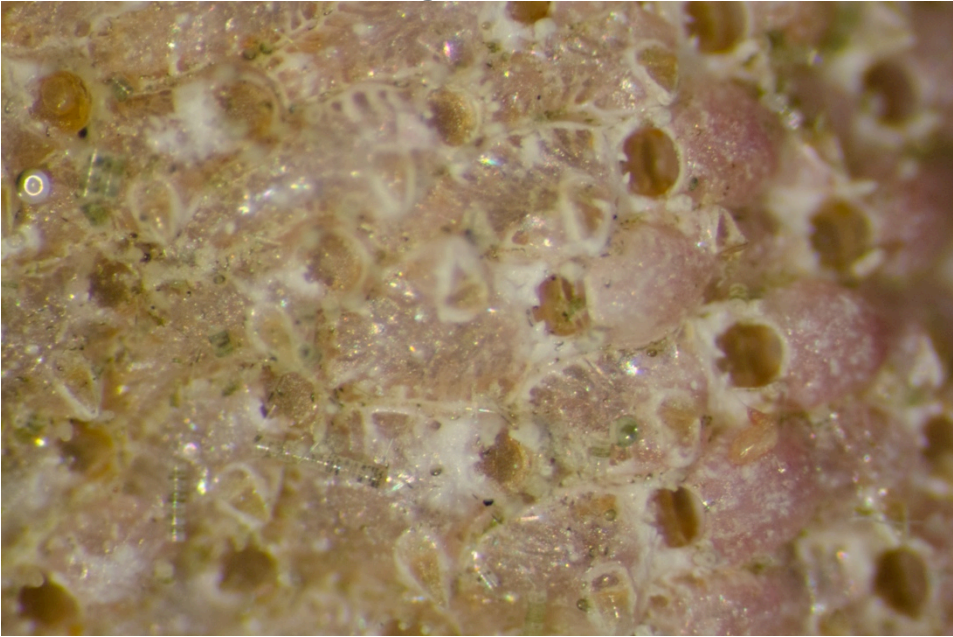
***Micropora notialis* Hayward & Ryland, 1993**



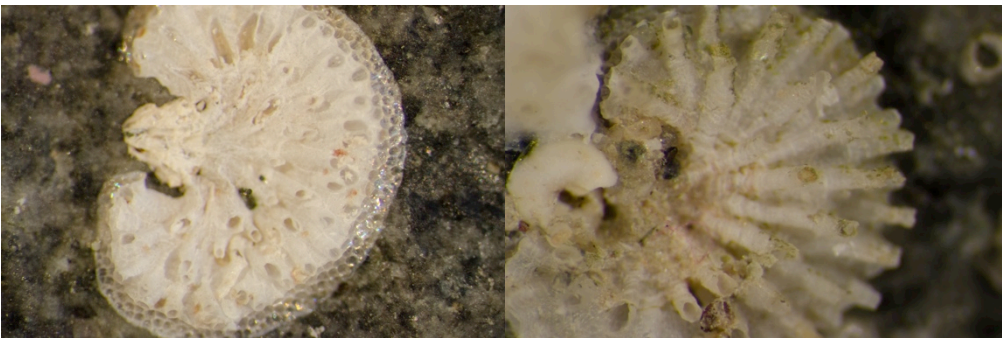
***Smittina* sp**



***Toretocheilum absidatum* Rogick, 1960**



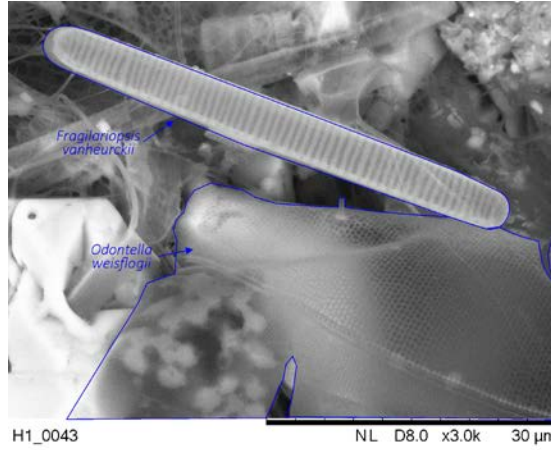
Class Stenolaenata
***Tubulipora* sp**



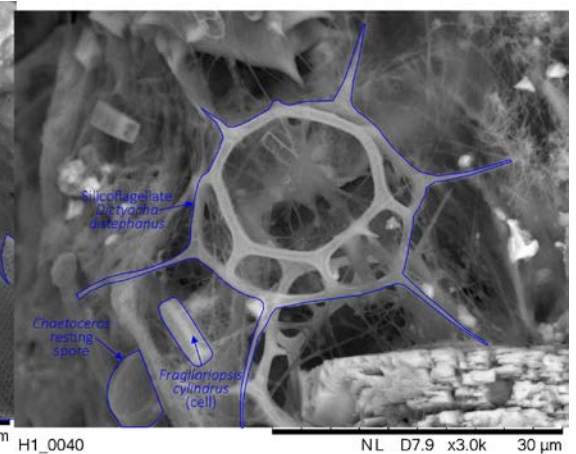
Appendix 2

Appendix 2 Supporting material for Chapter 5

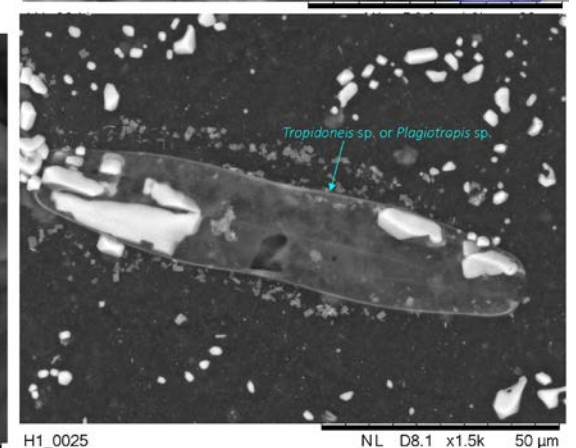
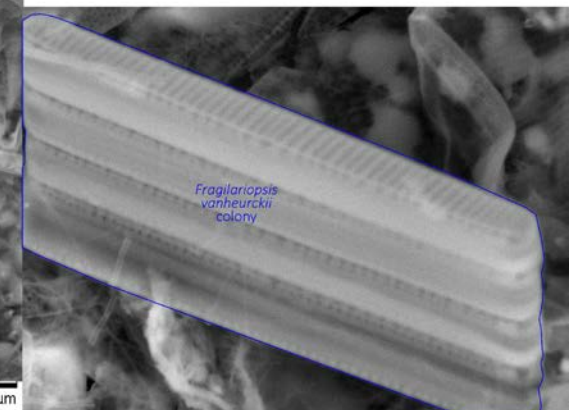
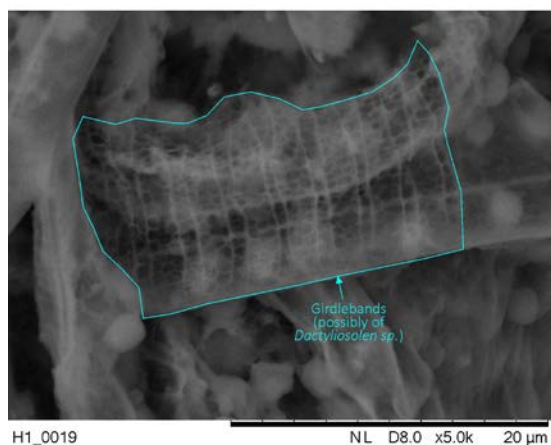
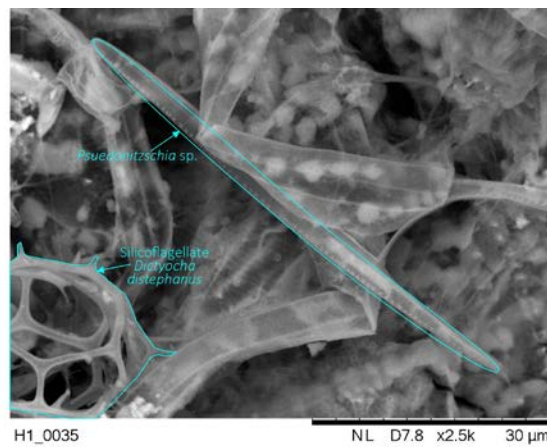
2.1 Photo identifications of diatom species found as prey items in the gut of the sea cucumber *H. steineni*. Identifications done by Southern Ocean Palaeoceanographer, Dr Clare Allen, The British Antarctic Survey



Magnification 8 x 3.0k 30µm
Top left: *Fragilariopsis vanheurckii*
Bottom: *Odontella weisflogii*

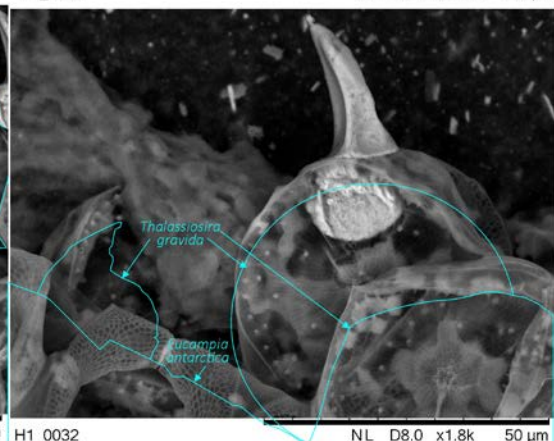
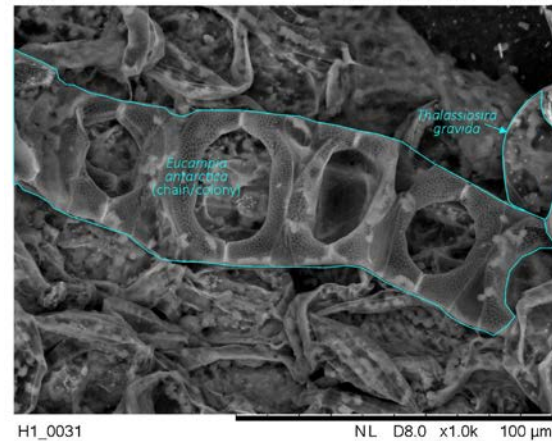
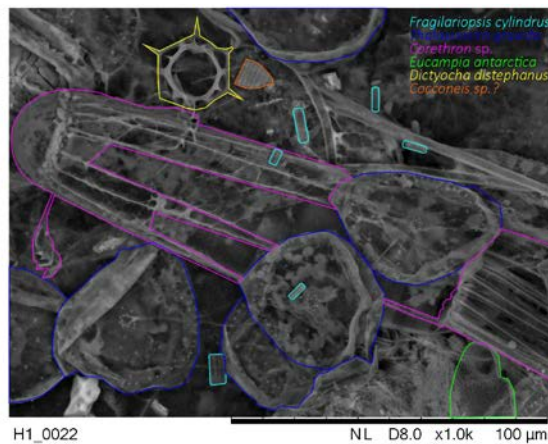
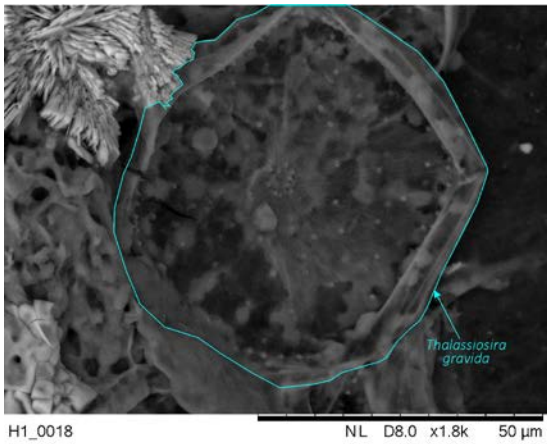


Magnification 7.9 x 3.0k 30µm
Central: *Dictyocha distephanus*
Bottom left: *Chaetoceros* resting spores
Bottom middle: *Fragilariopsis cylindrus*



Top left
 Magnification 7.8 x 2.5k 30µm
Psuedonitzschia sp
 Silicoflagellate, *Dictyocha distephanus*
 Bottom left
 Magnification 8 x 5.0k 20µm
Dactyliosolen cf girdlebands

Top right
 Magnification 8 x 3.0k 30µm
Fragilariopsis vanheurckil
 Bottom right
 Magnification 8.1 x 1.5k 50µm
Tropidoneis sp or *Plagiotropis* sp

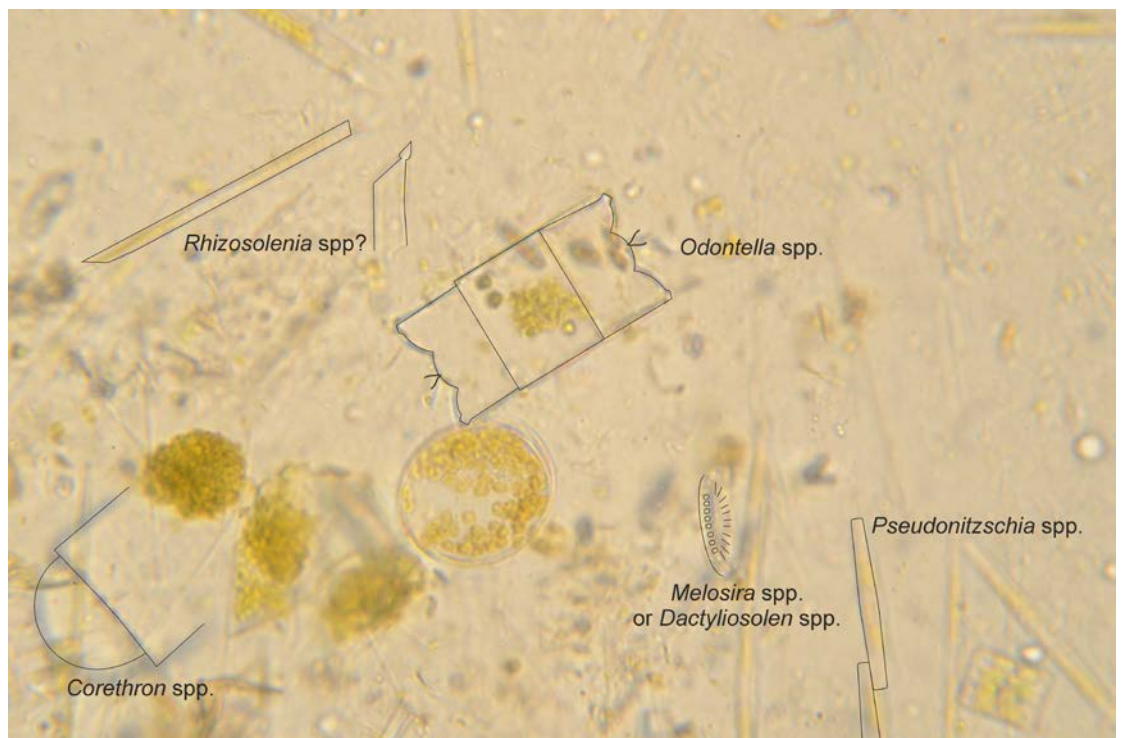
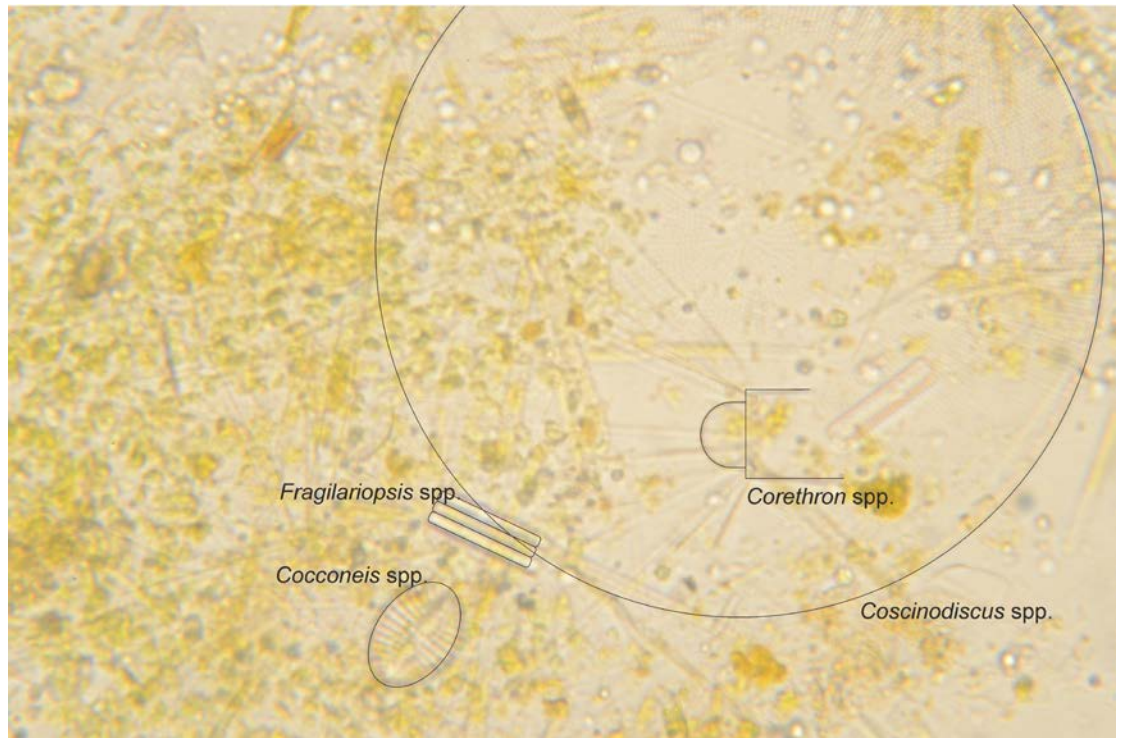


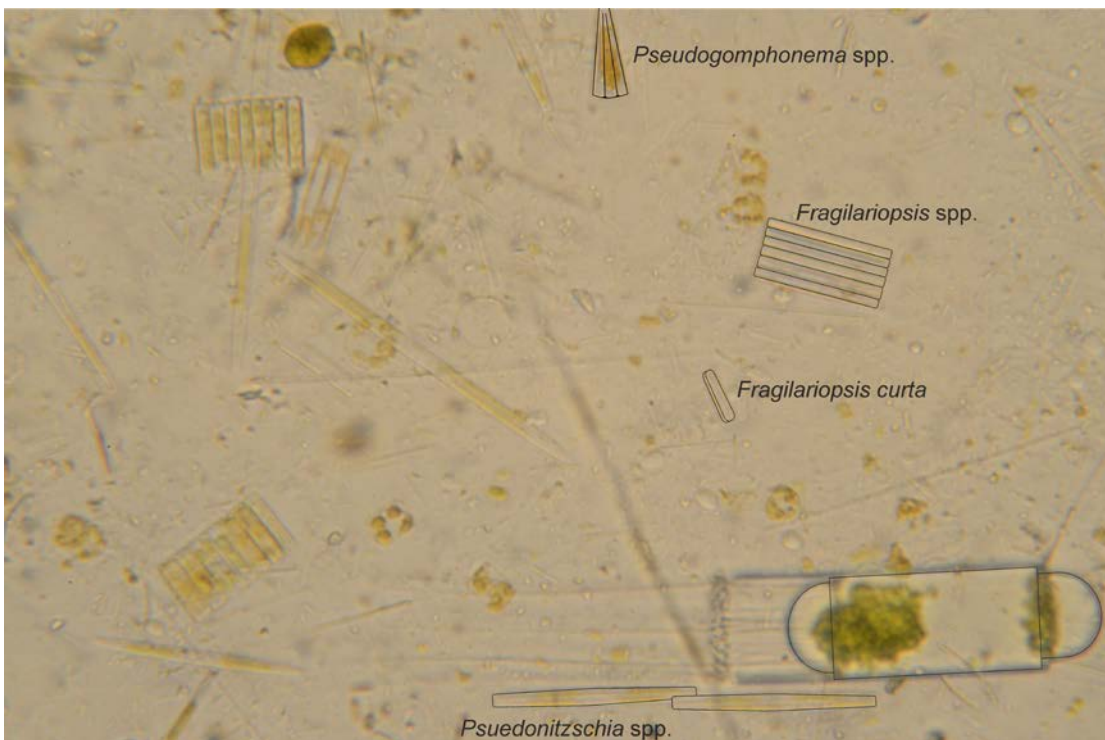
Top left
 Magnification 8.0 x 1.8k 50µm
Thalassiosira gravida

Top right
 Magnification 8 x 1.0k 100µm
 Yellow: *Dictyocha distephanus*
 Orange: *Cocconeis* sp
 Pink: *Corethron* sp
 Purple: *Thalassiosira gravida*
 Green: *Eucampia antarctica*
 Blue: *Fragilariopsis cylindrus*

Bottom left
 Magnification 8 x 1.0k 100µm
Eucampia antarctica (chain/colony)
Thalassiosira gravida

Bottom right
 Magnification 8.0 x 1.8k 50µm
Thalassiosira gravida



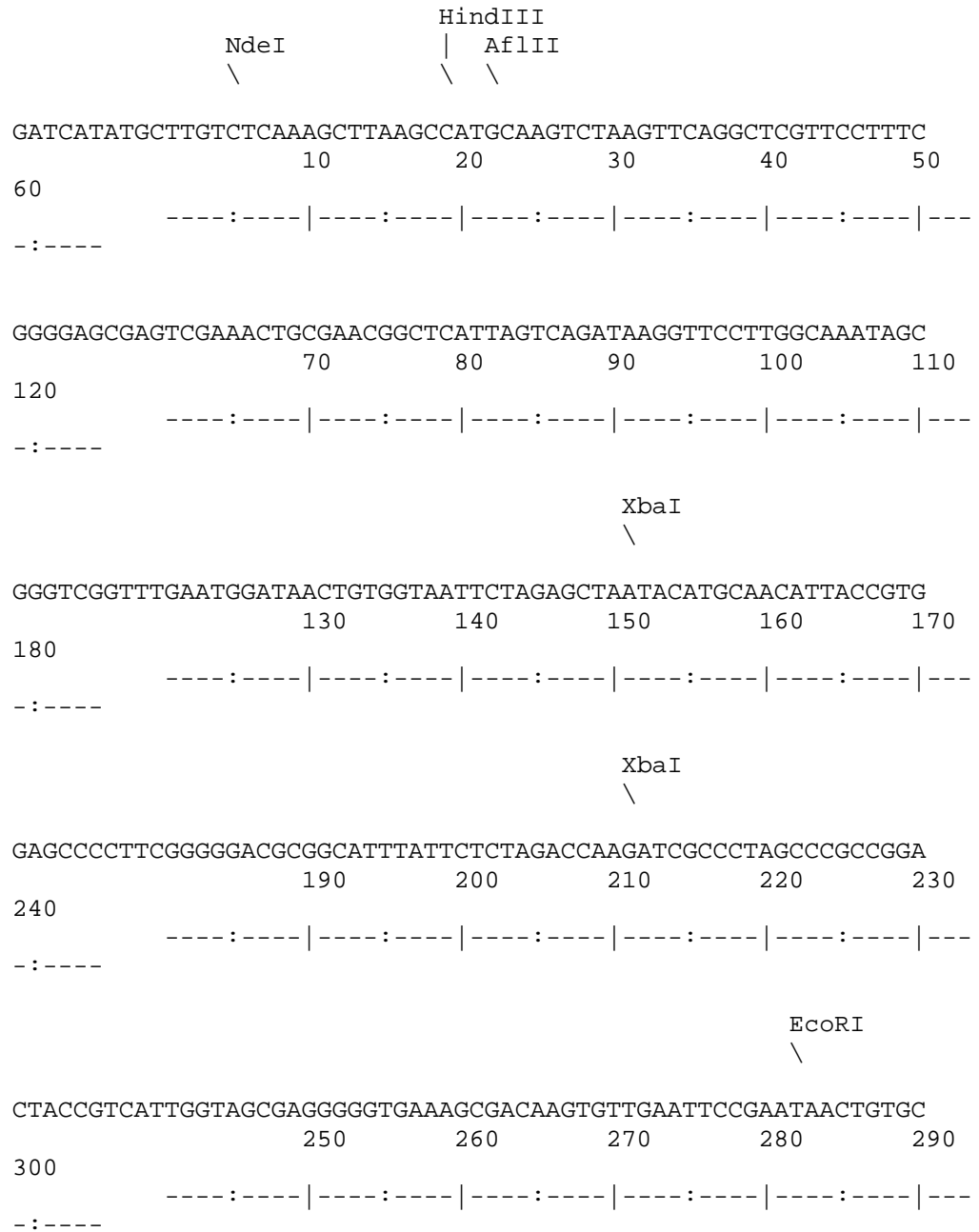


Light microscope at magnification x 50. Gut contents from the holothurian *H. steineni*, diluted with 96% ethanol for preservation and ease of viewing.

Appendix 2 Supporting material for Chapter 5

2.2 Genetic barcodes and restriction enzyme maps for the host species used to investigate marine invertebrate diets

*N. concinna*_18S



```

                                Cfr9I
                                | SmaI
                                | Bsp120I
                                | | ApaI
MfeI      PvuI
\          \ \ \
\
CGATCGCGGGGTCCACCCGGGCCCCCGACGACTTTGCCATGAAGTGTCTGTCCCATCAA
          310      320      330      340      350
360
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---
-:-----

                                BtgZI  BspMI
                                \      \
TTGGCGATGGTTCGGCGACCTGCCTACCACGGTGATGACGGGTAACGGGGAATCAGGGTTC
          370      380      390      400      410
420
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---
-:-----

                                BspMII*
BsePI
\
\
GATTCCGGAGGGGAGCCTGCGAAACGGCTACCACCTCCAAGGAGGGCAGCAGGCGCGCA
          430      440      450      460      470
480
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---
-:-----|

Bsp120I
                                EcoP15I
ApaI
\
\
ACTTACCCAATCCCGACACGGGGAGGTAGTGACGAAAAATAACGTGGCGGGGCCCTTACG
          490      500      510      520      530
540
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---
-:-----|

                                EciI
                                | BsrDI
BseRI
\          \
\
TGGTCGCCGCGGGCGGAATGAGCGCAATGTAAAAGATTGTGCGAGGAGCTATTGGAGGGC
          550      560      570      580      590
600
-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---
-:-----|

AAGCCCGGT
-----:-----

```

***O. validus* _18S**

```

                                NdeI                      EcoT22I      HindIII
                                \                          \          \
AGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTAAAAGCTTTTACAAGCG
      10      20      30      40      50
60
----:----|----:----|----:----|----:----|----:----|---
-:-----

AAACTGCGGATGGCTCATTAAATCAGTTATGGTTCCCTGGAGCGCACCCCCCTACATGGA
      70      80      90      100     110
120
----:----|----:----|----:----|----:----|----:----|---
-:-----

                                XbaI
                                \
TAACTGTGGTAATTCTAGAGCTAATACATGCCAGCAAGCGCCGACCTTGCGGGAGGCGTG
      130     140     150     160     170
180
----:----|----:----|----:----|----:----|----:----|---
-:-----

                                Cfr9I
                                | SmaI
                                \ \
CTTTTATTAGGAACAAGACCAACCCGGGCTCGTCCCGGCTCCGTTGGTGAACCTCTGGATA
      190     200     210     220     230
240
----:----|----:----|----:----|----:----|----:----|---
-:-----

                                PvuI
                                BbvII*
                                \
ACCTGGCCGATCGCACGGTCTTCGCACCGGCGACGGATCCTTCGAATGTCTGCCCTATCA
      250     260     270     280     290
300
----:----|----:----|----:----|----:----|----:----|---
-:-----

                                BamHI  AsuII
                                \      \
                                NcoI
                                \
ACTTTCGATGGTACGTTATGCGCCTACCATGGTCGTAACGGGTAACGGAGAATCAGGGTT
      310     320     330     340     350
360
----:----|----:----|----:----|----:----|----:----|---
-:-----|

```

BsePI	BspMII*	Bce83I*
\	\	\
CGATTCCGGAGAGGGAGCTTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGC		
	370	380 390 400 410
420	-----:----- -----:----- -----:----- -----:----- -----:----- ---	
-:-----		
	EcoP15I	
	\	
AAATTACCCACTCCCGACACGGGGAGGTAGTGACGAAAAATAGCAATACAGGACTCTTTC		
	430	440 450 460 470
480	-----:----- -----:----- -----:----- -----:----- -----:----- ---	
-:-----		
	AhaIII*	
	\	
GAGGCCCTGTAATTGGAATGAGTACACTTTAAATCCTTTAACGAGGATCTATTGGAGGGC		
	490	500 510 520 530
540	-----:----- -----:----- -----:----- -----:----- -----:----- ---	
-:-----		
AAGTCTGGT		
-----:-----		

O. victoriae_18S

```

                                NdeI                                EcoT22I
                                \                                \
AGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTCAGTACAAGTTTTTCTAAAC
      10      20      30      40      50
60
----:----|----:----|----:----|----:----|----:----|----
-:-----

GAAACTGCGGATGGCTCATTAAATCAGTCATGGTTCCTTGAACGAGTTGCCCTACATGG
      70      80      90     100     110
120
----:----|----:----|----:----|----:----|----:----|----
-:-----

                                XbaI                                Eco47III
                                \                                \
ATAACTGTGGTAATTCTAGAGCTAATACATGCCACCAAGCGCTGACCTTACGGAAAGCGT
      130     140     150     160     170
180
----:----|----:----|----:----|----:----|----:----|----
-:-----

                                Cfr9I
                                | SmaI
                                \ \
GCTTTTATTAGGAACAAGACCAACCCGGGCTCGCCCGGCTTCGTTGGTGAACCTCTGGATA
      190     200     210     220     230
240
----:----|----:----|----:----|----:----|----:----|----
-:-----

ACTTTGCAGATCGCACGGTCATCGTACCGGCGACAAATCCTTCAAACGTCTGACCTATCA
      250     260     270     280     290
300
----:----|----:----|----:----|----:----|----:----|----
-:-----

                                NcoI
                                \
ACTTTCGATGGTACTTTATGCGCCTACCATGGTCGTAACGGGTGACGGAGAATCAGGGTT
      310     320     330     340     350
360
----:----|----:----|----:----|----:----|----:----|----
-:-----

                                BspMII*
BsePI
\
\
CGATTCCGGAGAGGGAGCCTGAGAAATGGCTACCACATCCAAGGAAGGCAGCAGGCGCGC
      370     380     390     400     410
420
----:----|----:----|----:----|----:----|----:----|----
-:-----

                                EcoP15I
                                \
AAATTACCCACTCTCGACACGGGGAGGTAGTGACGAAAAATAACAATACAGGACTCTTTC
      430     440     450     460     470
480
----:----|----:----|----:----|----:----|----:----|----
-:-----
```

AhaIII*

\

GAGGCCCTGTAATTGGAATGAGTACACTTTAAATCCTTTAACGAGGATCTACTGGAGGGC

490

500

510

520

530

540

-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---

-:-----

AAGTCTGGT

-----:-----

***S. neumayeri* _18S**

```
XhoI
|   Esp3I
\   \
\   \
AGTCATATGCTTGTCTCAAAGATTAAAGCCATGCATGTCTAAGTACAAGCTCGTCTCGAGC
10      20      30      40      50
60
----:----|----:----|----:----|----:----|----:----|---
-:-----

GAAACTGCGGATGGCTCATTAAATCAGTTATGGTTCATTGGATCGAGTCCCCCGACATG
70      80      90      100     110
120
----:----|----:----|----:----|----:----|----:----|---
-:-----

XbaI
\
GATAACTGTGGTAATTCTAGAGCTAATACATGCGTCCAAGCGCCGACTTTCCAGAAGGCG
130     140     150     160     170
180
----:----|----:----|----:----|----:----|----:----|---
-:-----

XmaIII*
| MroNI
| | NaeI
| | | FseI
\ \ \ \

TGCTTTTATTAGGAACAAGACCAGCCCGGTTTCGGCCGGCCACACTGGTGAAGTCTGGAT
190     200     210     220     230
240
----:----|----:----|----:----|----:----|----:----|---
-:-----

PvuI
\
AACACAGCCGATCGCACGGTCTTTGCACCGGCGACGGATCCTTCGAATGTCTGCCCTATC
250     260     270     280     290
300
----:----|----:----|----:----|----:----|----:----|---
-:-----

BamHI
\
AsuII
\

NcoI
\

AACTTTTCGATGGTACGTTATGCGCCTACCATGGTCGTCACGGGCAACGGAGAATCAGGGT
310     320     330     340     350
360
----:----|----:----|----:----|----:----|----:----|---
-:-----

BspMII*
BsePI
\
TCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCG
370     380     390     400     410
420
----:----|----:----|----:----|----:----|----:----|---
-:-----
```

EcoP15I

\

CAAATTACCCACTCCCGACACGGGGAGGTAGTGACGAAAAATAACAATACAGGACTCTTT

430

440

450

460

470

480

-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---

---:-----

AhaIII*

\

BamHI

\

CGAGGCCCTGTAATTGGAATGAGTACACTTTTAAATCCTTTAACGAGGATCCACTGGAGGG

490

500

510

520

530

540

-----:-----|-----:-----|-----:-----|-----:-----|-----:-----|---

---:-----|

CAAGTCTGGT

550

-----:-----|

